T H E S I S

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"PHYSIOLOGICAL AND GENETICAL STUDIES IN THE POTATO (*Solanum tuberosum* L.) AND RELATED SPECIES".

by

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GENERAL INTRODUCTION.

There are a number of problems pertaining to the genetical investigation of the potato which have a definite physiological background. Work on a number of these intermediate problems was planned from the laboratory side.

On the one hand there are such questions as quality and the definition of "good" quality or "bad" quality. Precise definition of these with accurate methods of assessment would assist the geneticists. Part I of these studies deals with the question of quality with special reference to starch characteristics.

The problem of sterility or inability to produce viable progeny in its widest sense embraces in the potato a wide variety of causes, as for example :

Contabescent anthers.

"Bad" pollen, e.g., non-functional pollen or pollen capable of functioning in certain stigmas but not on others (possible oppositional factors, etc.)

Abscission of flower buds, flowers and fruits.

Causes/

Causes may be :

(a) cytological,

(b) physiological,

(c) genetical,

all complicated by relation to external factors. A physiological approach may be made to at least two of these, namely, abscission of flower buds, flowers and mature fruits; and to the problems of pollen germination and subsequent growth, both in vivo and vitro. These subjects are considered in Part II.

The problem of clonal selection coupled with peculiar gene production in the potato indicate that enquiry into possible chimerical structure would be of value to the plant breeder and, at the same time, have a physiological mode of approach. Part III of these studies is concerned with this aspect.

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PART I.

COOKING QUALITY OF POTATOES WITH SPECIAL REFERENCE TO STARCH.

C O N T E N T S.

INTRODUCTION.

The first step in any investigation of the starch involves preparation of the experimental material. In the literature a general method occurs and this involves :

(a) disruption of the cells of the tuber to release the starch grains,

(b) the washing of other material away, using screens, etc.,

(c) chemical treatments for improving the colour, and

(d) the separation of the starch from the water and its subsequent drying.

The literature dealing with the effect of heat on starch in water suspensions shows that the investigators used either dry starch prepared by themselves in this way or commercial samples. In some instances, but not in all, the chemical treatment was omitted.

Edwards & Ripperton (1933) ground or shredded the tuber and extracted the starch from the pulp through a fine screen or cheesecloth. The starch was further purified by repeated stirring, settling and decanting. The starch

was then air-dried over a hot-plate at a temperature not exceeding 40° C. Reichert (1913, 1919) used a similar method, but the starch was dried at 50° C. Arzichowski (1918) prepared the starch used in his investigation from one large tuber, washing the starch repeatedly and energetically before drying it. He found that many of the smaller grains were eliminated during the process of preparation. The method used in drying the starch is not stated, but it is plain that it was dried because, for the tests, he used a suspension of .05 gm. starch in 5 ccs. of water.

Francis & Smith (1916) steeped the material in water for several days until slight putrefaction was noticed. It was then crushed in a mortar and rinsed with water on a 100-mesh brass sieve until the rinsings became clear. The impure starch was then washed several times by decantation before being suspended in water to which enough 5% Sodium hydroxide solution was added to produce a yellow colour. This was vigorously stirred and set aside for six hours. The supernatant liquid was then decanted and the starch washed four times with water, once with

95%/

95% alcohol and finally with ether; then dried over Sulphuric acid in a desiccator. Dox & Roark (1917) used a similar method to this, but omitted the putrefaction of the material and the dilute Sodium hydroxide, being uncertain of their effect, and substituted alcohol. The starch was finally washed with ether on a hardened filter in a Hirsch funnel.

In the present investigation the starch was taken direct from the tuber on to a microscope slide, the drying stage being omitted. This procedure was adopted as there seemed no doubt that any form of pretreatment, such as heating and drying, must affect the subsequent behaviour of the starch grains, they being colloidal in nature and doubtless showing symptoms of hysterisis. In addition, the passing of the starch through screens or centrifuge was omitted and thus the possibility of damage to the individual grains was reduced very considerably. These simplified methods were designed to provide the closest approximation to what occurs when the potato is cooked and so give immediately applicable results.

Reichert (1913)/

Reichert (1913) gave a good description of the characters of potato starch from which the following has been extracted :-

"In form the grains are usually simple. There is no tendency for the grains to occur in clumps. The surface of the grains is rounded and quite smooth. Conspicuous shapes are ovoid, flattened ovoid, oval, and round. Some of the larger grains are slightly flattened. The hilum is distinct, small, usually round, non-refractive spot; situated usually at the larger end of the grain, often much to one side of the longitudinal line. The lamella are distinct, fine and, as a rule, regular in outline and tend to follow the outline of the margin. The lamellae average from 27 to 42 in number on medium sized and large grains. The grains vary in size from 15 to 70 μ. The common size is 44 μ".

Arzichowski (1918) gave the distribution of the size of grains from one large tuber as under :-

Length of Grains μ	Width of Grains μ					Total	Calculated According to	
	17 to 25	25 to 33	33 to 41	41 to 49	49 to 57		Curve of Pearsons Type I.	Gaussian or Normal Curve.
17 - 25	3	-	-	-	-	3	1.0	3.4
25 - 33	5	5	-	-	-	10	8.2	10.8
33 - 41	2	16	3	-	-	21	21.8	21.5
41 - 49	1	21	14	-	-	36	28.9	27.1
49 - 57	-	1	13	-	-	14	22.8	21.5
57 - 65	-	2	5	3	1	11	11.8	10.8
65 - 73	-	-	1	3	-	4	4.2	3.4
73 - 81	-	-	1	-	-	1	1.1	0.
Total	11	45	37	6	1	100		

Nagelli (1882) /

Nagelli (1882) found the length of the grains to be 70 - 90 μ. According to Payen (1838) the grains in the Rohan variety attain a length of 185 μ, and in some other kinds of potato 140 μ. Salaman (1926) stated, "the starch grains differ very much in size there being many gradations between the largest 100 μ x 71 μ and the smallest 30 μ x 30 μ".

The dimensions of starch grains are relatively easy to obtain and give approximations to the sizes of the grains. Berthault (1911) regarded one large grain as being 80 times the weight of a small one. Varieties ranged from those in which the starch consisted of 76% large grains to 24% small, through intermediate values, to the other extreme where some had 29% large to 71% small. The 71% of small grains corresponded only to 2.8% of the sample by weight. He found that the early maturing varieties were those with the largest number of big grains, the later maturing sorts on the contrary were made up of the smaller grains. Also when the tubers were matured there was no relation between size of tuber and relative proportion of big to small grains and this latter ratio had no relation to

the/

the total quantity of starch present. The matter of size of grain is of importance in the manufacture of starch as well as in cooking quality.

Numerous analyses of the composition of potatoes have been made by various workers and the following may be taken as an approxi - mation to the average :-

Water			78
Dry Matter :			
Starch ...	16		
Cellulose tissues	4		
Protein bodies	2	22	
		100	

The starch content of potatoes can be approxi - mately determined by finding the specific gravity of the tubers, since by far the greater part of the potato tuber is starch and water (94% approx.). The starch being much heavier than water, it is evident that the variation in starch content will affect the specific gravity of the tuber. The tables compiled by Parow mentioned in Salaman (1926) give the practical relationship. Grubb & Guilford (1913) make mention of a variation from 11.4 to 22.9% in the starch con- tent amongst some 77 varieties over a period of two years. This is approximately the same

range/

range as given by Johnson & Boyle (1918). Sperling (1909) and Dix (1923) found a high degree of uniformity in starch content of the tubers of different plants from the same variety and indicated that varieties may be grouped definitely into classes according to the percentage of starch present.

Grubb & Guilford (1913) again mention that a test of different sized tubers of the same variety proved that there was practically no difference in the starch content of large and small tubers. However, there were large variations between the starch content of tubers from different soil depths, being largest in the deeper growing, and it was suggested that the 'depth influence' acted through the temperature of the soil, the deeper tubers being in a cooler medium than the shallow ones.

The quantity of starch present in the cells of various portions of the potato tuber differ not only from one another but also between different varieties, an average figure for each portion being :-

Periderm	... Nil
Cortex	... 26%
External pith	14
Internal pith	7

Lehmann (1926) /

Lehmann (1926) working on the anatomy of the potato tuber found that there existed a positive correlation between the size of the tuber and the size of the cells composing it; large tubers are always made up of larger cells than smaller tubers. According to Bredemann & Schulz (1931) the size of potato tuber cells, although affected by various environmental conditions, were more or less characteristic for each variety. There was no correlation between size of cells and starch grains (presumably size of grains), nor between cell sizes and time of maturity. Varieties also differed in the proportion of different size cells and starch grains. Nitrogen manuring caused an increase in the mean cell size by reducing the proportion of smaller cells and increasing the proportion of larger cells. Potassium increased cell size when compared with "No potassium". Previous annual applications of Nitrogen or Phosphate exerted a residual effect on cell size. Cell size was, therefore, regarded of practical importance in starch manufacture, because the larger the cells the larger the yield of starch.

There are few references in the literature to methods for obtaining the specific gravity/

gravity, physical condition (moisture content, size, weight), etc. of the starch grains. The estimates of specific gravity of potato starch will be influenced greatly by the amount of moisture present in the starch. Fluckiger (1891) gave the specific gravity of potato starch as 1.503. When completely dry 1.633. Parrow (1908) gave the density of potato starch as 1.648. Harvey (1924) using the vacuum jacketed pycnometer found the specific gravity of potato starch to be 1.497 and the specific volume to be 0.667. Gruss (1932) secured the separation of large and small starch granules of barley by :-

(a) a systematic sedimentation, aided in some cases by centrifuging, and

(b) counts of ganules under the microscope.

He found the specific gravity of the large and small sized starch granules of barley to be 1.526 and 1.144 respectively. Gertz (1922) stated that potato starch contains a large amount of water, up to 40 - 50%, and dry potato flour of commerce contains 19.22 - 19.88%. The method used for testing the amount of water was to heat the starch in a liquid which did not absorb water, as Paraffin Oil, to about 120° C. He left it undecided whether the water vapour produced originated wholly from merely mechanical held

water/

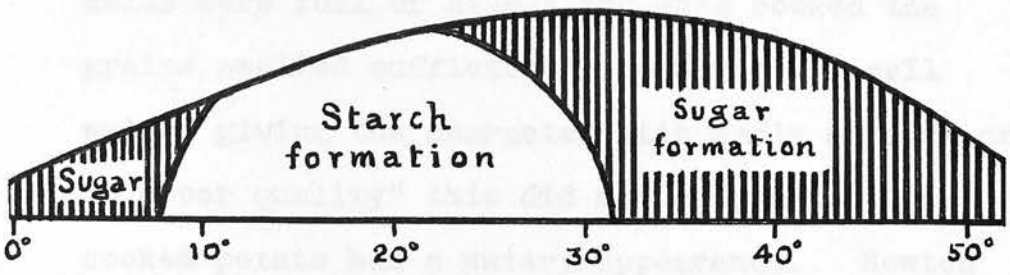
water or otherwise.

In common with other starches potato starch grains are optically active and if examined in water under a polarizing microscope they show a distinct "cross". This polarizing figure or "cross" consists of four fairly clear cut lines radiating from the hilum. When the starch grains are heated in water to the temperature of gelatinization, it will be found that the process of swelling and gelatinization can be accurately traced by the disappearance of the "cross". This method of securing a sharp gelatinization point has been used by Reichert (1913, 1919), Francis & Smith (1916), and Dox & Roark (1917), who recorded the temperature at which there was a disappearance of anistropy of all, or practically all, starch grains. Arzichowski (1918) used the same method for recording the condition of individual grains which he classified as swollen, semi-swollen or not-swollen.

Conditions of storage (particularly temperature) affect the starch of potato tubers, which fact has been shown by several workers. Butler (1920) found that sugar accumulated rapidly

in/

in potatoes stored at 0° C., and at relatively slower rates at temperatures up to 10° C. For culinary purposes a storage temperature of about 7.8° C. was recommended. Wolff (1926) stated the sugar ⇌ starch equilibrium over a fairly wide range of temperatures and showed the effect of temperature diagramatically as under :-



Sweetman (1931) stated that potato storage temperature below 7.5° C. caused undesirable accumulations of sugar. Richardson & Douglass (1929) reported that although storage in a humid cellar at 4.5° C. might be desirable for potatoes to be used for seed, storage in a dry cellar at 11.7 to 15.6° C. was superior for those to be used for cooking, because such tubers were much more mealy.

There is a general opinion that the cooking quality of potato varieties differ and that climatic and soil conditions greatly affect

this/.

this. Standards of quality, however, are difficult to arrive at and will vary in many respects according to personal taste and method of cooking. Newton (1920) has stated that Canadians prefer mealy white-centred potatoes, and found that the mealy appearance of cooked potato was due to the peculiar starch content of cells. In such "good quality" potatoes the cells were full of starch and when cooked the grains swelled sufficiently to burst the cell walls, giving the characteristic mealy appearance. In "poor quality" this did not happen and the cooked potato had a watery appearance. Newton further found no relationship between tuber shape and culinary quality, but in general, round flat or oblong flat types were best, these flatter types seeming to be easier to cook right through. Comparison of probable cooking quality could be estimated, so this writer suggested, by holding to the light thin sections cut through the middle. In all tubers the central tissue had the lowest starch content and if this area is very small the quality should be high. In potatoes of low quality this central area was transparent due to the high water content and the greater the amount of watery tissue the

poorer/

poorer was the quality. With regard to soils the light sandy loam was preferred. In peat soils the rapid growth of tuber tended to make it watery. A long growing season was essential and a significant proportion of starch was deposited in tuber tissues during the last stages of growth. Parker (1932) doubted the accuracy of public taste where quality of potatoes was concerned. Among First Earlies a waxy (but not soapy) texture was required. In the crop of later harvesting the main requirement was that the flesh should be white, that the minimum of discolouration should be produced when the potatoes were boiled or steamed and then reheated, and a floury texture was demanded by a vast majority of consumers. Tests carried out by the National Institute of Agricultural Botany in co-operation with J. Lyons & Co. Ltd., indicated :-

(1) Little difference in the tests at different times.

(2) Amongst varieties already popular soil differences were greater than differences in variety.

(3) Colour/

(3) Colour after cooking was influenced by soil rather than by variety.

(4) Flavour was a varietal rather than an environmental character.

Sweetman (1931) studied the factors affecting or correlated with mealiness when potatoes were boiled or baked. The factors examined were amount of starch, size of starch grains, viscosity of the starch, increase in the volume of the starch during heating, content of soluble pectin and protopectin and amount of sloughing when boiled. No one of these factors was correlated completely with mealiness, but it was hoped that it might be possible to locate the combination of factors which would explain the lack of mealiness in certain tubers of high starch content. Sweetman (1933) found that cooking of potatoes did not cause the bursting of cell walls, but permitted ready mechanical disintegration of the starch, solution of some of the pectic substances, increased digestibility of the cellulose, coagulation of most of the protein, and some degree of caramelization of the sugar. No supporting figures were given. Grubb & Guilford (1913) stated that quality could

be/

be definitely tested only by cooking. This test was best made by boiling in the skins or baking. After removing from the fire the tuber was held in a napkin and squeezed lightly, then broken open. If the starch was abundant a white flaky mass somewhat shiny and crystaline in appearance resulted. If the starch was scanty the tuber would be soggy and might have a watery core. Peacock & Brunstetter (1931) advocated the picric acid test for pre-determining the culinary quality of potatoes for chip making, french frying, baking and, under certain conditions, for boiling. The picric acid test determined the content of soluble sugars which, in high grade potatoes, should be very low.

Salaman (1926) in his description of varieties gave relative cooking quality values derived from repeated tests on tubers which had been cooked whole under steam. Texture of the cooked potato was defined as :

(a) "Floury" - the tubers burst spontaneously, or on the application of a fork break to pieces and crumble. The broken surface often glitters in the light.

(b) "Close" - the tubers do not burst, but readily break to the fork without

crumbling/

crumbling.

(c) "Waxy" - the flesh is firm and consistent and only breaks down by definite kneading.

"Soapy" - the consistency is the same as "waxy", but the flesh appears watery and somewhat translucent.

Taste (largely a question of individual liking) was described by Salaman in the terms :

(1) Strong or Strong and pleasant.

(2) Mild and/or Pleasant.

(3) Tasteless or Insipid.

The following table has been prepared from his varietal descriptions :-

"Floury" Texture.

Strong Taste.	Mild Taste.	Taste not stated
Abundance Crimson Beauty Edzell Blue	Arran Comrade Champion Kerr's Pink Langworthy Snowdrop.	Arran Rose Arran Victory British Queen Crusader Reading Russet

"Floury - Close" Texture.

Strong Taste.	Mild Taste.	Insipid.
Flourball.	Arran Chief Cardinal Golden Wonder Great Scot Katie Glover.	Majestic President

"Close" Texture.

Strong Taste.	Mild Taste.	Insipid.
Up-to-Date	Catriona Duke of York Epicure King Edward Edgecote Purple	Adirondack

"Close - Waxy" Texture.

Strong Taste.	Mild Taste.	Insipid.
-	Sharpes' Express	Ally * Templar

"Waxy" Texture.

Strong Taste.	Mild Taste.	Insipid.
Eclipse	Bishop Di Vernon	Evergood * Mr Bresee *

* = "Soapy".

Russell (1933) commenting on the effect of manures on the cooking quality of potatoes, stated that as regards the potassic salts the boiling quality was injured by chlorides; Sulphate of Potash gave the best quality, Kainit the poorest. He quoted the marks given for quality of steamed potatoes with Woburn and

Rothamsted/

Rothamsted material from soils treated with Potash and with Nitrogenous fertilisers.

Dose.	Nitrogenous Fertiliser.		Potash Fertiliser	
	Woburn	Roth'sted.	Woburn	Roth'sted.
0	34.4	29.2	32.6	28.5
1	33.3	29.3	33.6	29.5
2	32.9	29.1	34.5	29.6

Gozsy & Meszaros (1931) investigating the relationship of the chemical composition to the flavour, cooking and keeping properties, examined 10 varieties of potatoes which had been grown under similar conditions. Potatoes showing a low starch content and a high Nitrogen content were found in general to have the best taste and keeping qualities when kept dry or in pure or saline water. No correlation was established between chemical composition and fat absorption. The chemical and other determinations made were for starch percentage, Nitrogen (Protein) percentage, specific gravity of the tubers, dry matter percentage (by specific gravity calculation), and dry matter percentage by weighing. The cooking investigations

consisted/

consisted of :

(a) Palatability tests in which the varieties were placed in order of preference for seven different methods of cooking.

(b) Time tests for cooking to the soft and to the mashed condition.

(c) Determination of the percentage of fat absorbed.

Neil & Whittemore (1930) after a four year study of the relation between mealiness in potatoes and the amount of Potash in the fertiliser, found that boiled mashed or baked potatoes were more mealy when fertilised with a high quantity of Potash. The Muriate and Sulphate of Potash seemed to be of equal value in producing a mealy potato. In this study they found that the percentage of starch did not seem to affect mealiness, the more mealy potatoes in several seasons contained less starch. Sinoda, Kodera & Oya (1931) working on the chemical changes in the carbohydrates in the sweet potato according to methods of cooking, namely, boiling, steaming or baking, found a decrease in soluble sugars and dextrins, but the starch fraction was

increased/

increased. Sinoda & Kodera (1932) found that the lowest temperature at which the sweet potato could be cooked was between 70 and 80° C., and coincided with the gelatinization temperature, which is 74° C. for this material.

The effect of heat on starch in water suspensions, the amount and rate of swelling of the individual starch grains, and the changes in viscosity of the suspensions at various temperatures, etc., have been investigated by a number of workers. Wiegel (1933) studied viscosity changes and found in the temperature distribution curves a sharp maximum at 60° C. The starch was given pretreatment with warm water at 50 to 60° C. Reich & Damansky (1933) found that the "viscosity curve" of the natural starches corresponded more closely to that of the amylopectin (or outer envelope of the starch grains). In the temp-viscosity diagram for the potato starch suspension they showed a sharp maximum at 57.9° C. They also concluded that the point of gelatinization for grains of the one size depended largely on the actual size and the density of the grains. Thurber (1933) determined the

temperature/

temperature of gelatinization of potato starch by noting the temperature at which increase of the viscosity of suspensions in water occurred. His figure for potato starch was 61° C. Ripperton (1931) and Wiegel (1933) used similar methods in studying the effect of electrolytes on the heated starch suspensions.

At the best, viscosity measurements give only the gross effect of the mass of starch grains making up the preparation. Much use has been made of the microscope in studying the swelling of the starch grains and many investigators have attempted to determine what is known as the Gelatinization Temperature or Gelatinization Range. The methods adopted and the materials used correspond more or less with that used in viscosity measurements, namely, standard suspensions of prepared starches submitted to a steadily rising temperature, the time factor increasing in proportion to the temperature. Lippmann (1861) put potato starch and water in a beaker in a water bath which was slowly heated. The preparation was subjected to microscopic examination at proper intervals. He found that swelling began at 46.25° C., gelatinization at

58.75° C./

58.75° C., and that gelatinization was complete at 62.5° C. Whymper (1909) recorded gelatinization temperatures by the "gradual - rising-temperature" method. The larger granules of any given starch were found almost invariably to succumb more quickly than the smaller grains to both wet and dry heat, and to diastase and mineral acids. It varied also with the state of maturity of the grains. Nyman (1912) did not find so much difference in the gelatinization temperature, but noted a decided difference in the time required for gelatinization at a given temperature. Reichert (1913) stated that the gelatinization temperature of potato starch was 65 to 67° C., mean 66° C. His method was to place a little of the starch suspension in a test-tube in a water bath, the temperature of which was raised very slowly and the water occasionally stirred. Specimens of the starch were examined at intervals the tube being shaken and the specimen obtained by inserting the end of a pipette to the bottom of the tube. Each specimen was placed on a slide and examined in the polarising microscope. The temperature at which there was a disappearance

of/

of anistropy of practically all the grains was recorded as the temperature of the tube. The water bath was at a slightly higher temperature as compared with the tube, and the actual temperature of gelatinization lay somewhere between the two and for convenience, especially for purposes of comparison, the mean of the two was for obvious reasons taken as the temperature of gelatinization. Reichert considered the use of polarised light very desirable in studying the gelatinization and swelling of starch grains, as the progress of the process can accurately be traced by the corresponding disappearance of optical activity. Francis & Smith (1916) found that the gelatinization temperature varied with the rate of heating. Their apparatus for the determination of the gelatinization temperature consisted of a thermo-slide, the temperature of which was started 3 - 5° below that found by preliminary test of the disappearance of optical properties in the material under examination. The temperature was then slowly raised and a record made of the temperature indicated by the thermometer in the thermo-slide, when the observer could no longer see any signs of anisotropy. By repeating the determination it was found possible

to measure the gelatinization temperature to within a few tenths of a degree. It was found important to have the starch well covered with water during the test, because if the water was allowed to evaporate, gelatinization might not occur or might be delayed for several degrees. The effect of lack of water was observed on potato starch (which, according to their determinations gelatinizes from 67.4° to 67.8° in the presence of water) in an experiment during which the samples were covered with glycerine and heated to 100° without any apparent action on the granules. The test must not be prolonged because high results may be obtained, due to the fact that some granules may partly gelatinize and the resulting paste protect the remainder from the action of the water. When using the water-bath test-tube method (Reichert (1913)), it was noticed that Arrowroot starch heated from 65 to 79° C. did not entirely lose its anisotropic condition at the higher temperature, but when the sample was plunged into water heated to 75° C., the starch lost its polarising properties immediately. In other words, it appeared that, if starch was subjected to a gradual rise of

temperature/

temperature in the presence of water, the gelatinizing point may become indefinite and not so uniform as when the starch was heated for a few minutes within 3 to 5° of the true point of gelatinization. In the thermo-slide method the starch was constantly under microscopic observation, and each test required less than five minutes to complete, the rise in temperature being of the order of 1° per minute. For potato starch the gelatinization temperature determinations were :-

(a) by the thermo-slide method 67.6
67.8
67.8
Aver. = 67.7° C.

(b) by the water-bath test-tube method ... 67
68
Aver. = 67.5° C.

Dox & Roark (1917) used an electrically heated chamber (an adaption of an electric incubator) on the microscopic stage. The starch preparation was placed on a thin hanging drop slide with the proper amount of water sufficient to prevent drying out. In operation they started at 5° below the previously noted gelatinization temperature and increased the

temperature/

temperature at the rate of 1° per minute. By this means they studied thirteen different varieties of Maize starch and found significant differences. The variation in temperature of different parts of the chamber was corrected by observing the melting of three organic substances, whose melting points covered the gelatinization temperature range. These substances were put under the same conditions, except that water was omitted. They found that the apparent gelatinization temperature had to be reduced 3.8° C. to give the actual gelatinization point. Arzichowski (1918) studied the swelling of starch granules microscopically, the material being obtained from one large potato of a local variety. He used an Ostwald Thermostat maintained at a constant temperature to an accuracy of 0.1° C. The prepared slides were placed at the bottom of a special vessel and immersed in the thermostat for two hours in some series of experiments, and 40 minutes in others. The vessels were afterwards taken out, the quickly cooled preparations investigated under the microscope and the starch grains classified into three categories, namely, completely swollen, semi-swollen and not-swollen.

The/

The refraction of light was used as the basis of judging the three states. From the moment when there appeared a first section of the grain giving weak refraction to the moment when the last area with high refraction disappeared, he considered the grain as semi-swollen, no matter how small was the area giving the different index of refraction. He defined the following exceptions :-

(1) Grains in which groups of crystals for a long time remained unswollen. These groups drew together and prevented the regular sac or bag shape. Under the microscope these appeared as black needles. These grains, he considered completely swollen, even though they contained some unswollen substance.

(2) Some grains had a black dot, but no areas giving weak refraction. He regarded these as unswollen.

The classes completely swollen and not-swollen were definite and needed no comment. His conclusions were :-

(1)

(1) that the process of gelatinization of potato starch (from this one tuber) occurred through a wide temperature range of not less than 12.5° from 55 to 67.5° C.

(2) that it was impossible to determine the temperature point of gelatinization, but the mean temperature of gelatinization could be determined with considerable accuracy.

(3) That the mean temperature of the beginning of swelling of potato starch grains in the most exact series of his experiments was found to equal 59.61 ± 0.10° C., and the mean temperature of complete swelling in which the starch grain did not contain any intact areas which gave strong refraction in the same series was found to be 60.97 ± 0.10° C.

(4) that the variation of character of starch grains observed during the process of gelatinization was completely similar to the

fluctuating variation of organisms.

(5) that the size of grain and the rapidity of heating exercised a considerable effect on the process of gelatinization. Smaller grains withstood (i.e. were more resistant to) the effect of heat more than large ones and slow heating was tolerated better than rapid heating.

Huss (1922) studied the swelling of starch grains when stained in dilute solutions of certain specified stains, namely, methyl violet, methylene blue, thionin, Bismarck brown, malachite green, methyl green, safranin. The starch grains were not stained in dilute solutions of eosin, congo red, brilliant blue, water blue, phenol red and azolitmin unless through mechanical pressure by heating in water until the initial state of swelling was reached, or through chemical reagents which change their structure, then these stains were absorbed. It was often more convenient to use these stains instead of polarised light in studying the swelling of the grains. Some instances were quoted where the

progress/

progress in swelling of the grains in hot water was studied with congo red.

Alsberg (1926) found that starch granules when heated in water swell, but do not burst, although potato starch granules do disintegrate to some extent. In investigating the swollen granules he noted that they appeared to be composed of a sac-like structure filled with a fluid. When tannin was allowed to diffuse into such swollen granules it formed particles which exhibited strong Brownian movement, indicating that the interior of the granules was either a sol or a solution, not a gel. He noted three factors as being responsible for the gelatinization of starch by heat :-

(1) The ease with which the anatomical structure was softened by moist heat.

(2) The inherent swelling power of the granule substance.

(3) The relation of the mass of the swelling substance to the surface area of the granule.

The larger the granule, the greater was the mass of the swelling substance which exerted pressure on a unit area of the granule surface. Therefore, a large granule would swell more rapidly

than/

than a small one, and it should swell sooner when the temperature was raised. He estimated that potato starch granules increased 40 times in volume during gelatinization. He concluded that there was no such physical constant of starch as a gelatinization temperature, but only a gelatinization range of temperature. In the case of potato starch, swelling began below 50° C. The size of the granules increased as the temperature was increased and did not reach its maximum until just below boiling. The process of swelling was a gradual one proceeding over a range of at least 50° C. Sweetman (1933) remarked that if complete gelatinization was coincident with maximum swelling, it was obvious that the starch present in an average potato in a ratio of 1 : 6 of water was not completely gelatinized during cooking. This was perhaps indicated by the smaller degree of transluscence which developed in a cooked potato as compared with that of potato starch pastes. The temperature of the interior of the tuber at "done-ness", which varied with tuber size and was about 94° C. in a 6-oz. potato cooked by boiling, might or might not be a limiting factor in gelatinization.

::::::::::

MATERIAL and METHODS.

The material consisted of tubers derived from several sources, namely :-

(a) Plants grown under field conditions.

(b) Plants grown in 10" pots.

Included in the latter were plants from the operational work described in Part III of this paper, and sets which had been given a range of eight manurial treatments comprising Superphosphate (P), Sulphate of Potash (K), Sulphate of Ammonia (N) alone, and in all combinations, from none to a dressing of all three. The treatment of the seed tubers, soil conditions, watering, etc., are described in Part II. After harvesting, which was performed when the plants had died down, the tubers were marked, weighed and stored in a cool dark place until used.

A number of the tubers were used for a test of the specific gravity as affected by variety, manurial treatment, and the operational work of Part III. The method used consisted of immersing the marked tubers in a

series/

series of salt (Sodium chloride) solutions, the specific gravity of which had been very carefully adjusted by means of a hydrometer. The solutions used and their approximate salt content (percentage by weight) are given :-

Specific Gravity of Solutions of Sodium chloride.

Specific Gravity.	Per cent. of Salt
1.03	4.2
1.04	5.6
1.05	6.9
1.06	8.2
1.07	9.6
1.08	10.9
1.09	12.2
1.10	13.5
1.11	14.8
1.12	16.1
1.13	17.4
1.14	18.8

The tubers were immersed in the weakest solution first. This should be of a strength so that all the tubers sink. (If any float it is necessary to make still weaker solutions until the range had been covered). Then the tubers were taken out, allowed to drain for a few seconds, and immersed in the next stronger solution. Those which sank to the bottom were then transferred to the next stronger

solution/

solution and the process continued until there were none left "sunk". The strengths of the solution in which the tubers first "floated" were taken to indicate their specific gravities. The marking of the tubers was done with an indelible pencil, and this was very effective, as the salt solutions did not cause the marks to "run". Additional intermediate strength solutions could be employed, but a limit would soon be reached, the tubers in most cases floating at varying depths instead of "sinking" or "floating". The depth of the vessels would then become the important factor. It was observed that little significant change took place in the strength of the solutions during use.

In the investigations of the effect of heat on starch it was decided to determine firstly the reaction of the starch taken practically direct from the tuber to the heating chamber where the temperature was at one of various levels and maintained constant. The observations were made using a low power objective in the microscope, and the eyepiece fitted with a disc marked in squares with sides corresponding to 100 μ and, when necessary, a polarising

attachment/

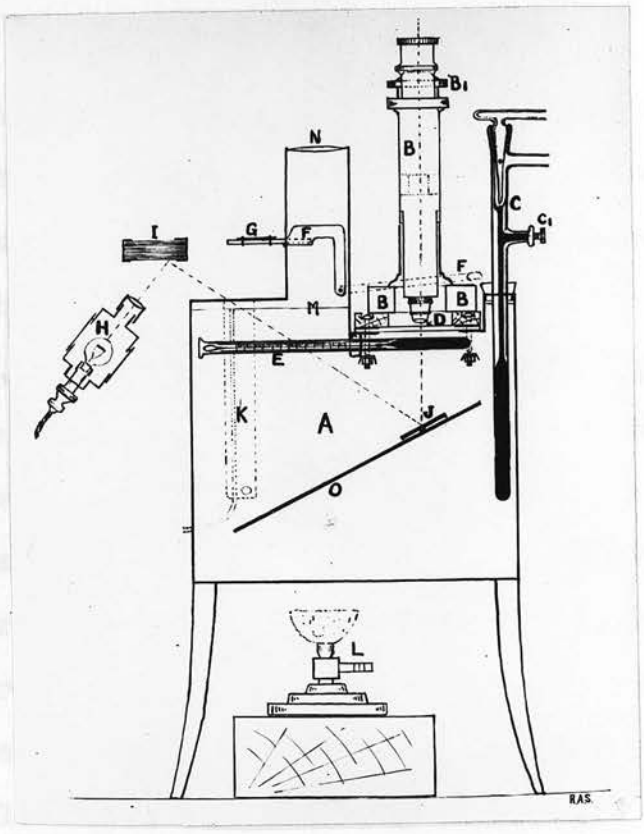


FIGURE 1.

A. Tank containing water.
B. Microscope and stand.
B_1 Fine adjustment of microscope.
C. Thermo-regulator.
C_1 Adjustment of thermo-regulator.
D. Piece of thin plate glass clamped to tank.
E. Thermometer.
F. Arm for carrying slide and sample into water and under the microscope.
G. Cell containing starch sample.
H. Microscope lamp.
I. Block of plates for polarizer.
J. Mirror for microscope.
K. Constant level device.
L. Drip-proof burner.
M. Level of water in tank.
N. Lens for reading temperature.
O. Plate for regulating circulation of water.

attachment was added. The draw-tube of the microscope was kept fully extended during the observations. For heating the sample of starch an electric microscope hot stage was tried, but discarded owing to difficulty of determining the temperature of the starch sample. The evaporation of water from the slide and the supersensitive regulator of the thermostat for different temperatures were other undesirable features. The apparatus used was designed and constructed by the writer and provided :-

(1) a means of securing a constant temperature,

(2) a considerable bulk of water at the desired temperature so that there would be an insignificant change in temperature when the sample was put in.

(3) devices for keeping the water at a constant level and for stirring it by regulated thermal circulation,

(4) the thermometer in close proximity to the starch sample and in the same stream of hot water,

(5) /

(5) a device for bringing the sample of starch quickly into the hot water and into position under the microscope,

(6) efficient lighting of the object, with or without polarised light, and

(7) timing by means of a stopwatch.

The heating was done with gas, controlled by a sensitive mercury thermoregulator of the usual design placed as close as possible to the sample and to the thermometer. The thermometer was specially constructed to take up a minimum of space in the tank and give readings to 1/10° C. over a range from 49 to 71° C. An expansion chamber was provided at the top of the thermometer to avoid damage if the water was allowed to boil by accident. The source of polarized light was a block of about thirty thin glass plates placed in a suitable position to reflect a powerful beam of light to the mirror beneath the microscope. When the apparatus was ready and the water at the desired temperature the starch sample was prepared from the tuber. This operation consisted of taking a

very/

very small core from the tuber by means of a fine tube and scraping a number of starch from the core at any desired tissue-depth directly into a drop of water on the slide. The tuber was placed in a moist atmosphere and left for 24 hours to ensure healing of the small wound made by the removal of the core. The cover-slip, carefully standardised for thickness and size, was placed on a rubber ring encircling the drop containing the starch, and kept in place there by two small rubber bands. This made a cell containing the sample and it was now ready to be swung into the hot water and under the microscope, where it would be upsidedown. The starch grains having a specific gravity of approximately 1.5 would be resting on the cover-slip and with only 1/10 mm. of glass between them and the hot water. Furthermore, the water in the small cell containing the starch would be heated from all sides, and especially through the thin coverglass, and thus quickly reach the desired temperature. The time lag for the cell to reach the given temperature was less than 15 seconds, and for the starch grains certainly less than 5 seconds. The slide automatically took up a position in focus

under /

under the microscope which was quickly moved to examine the whole cell and thus to permit a suitable field being selected for observation. The stopwatch was started at the same time as the slide was swung into the water. Observations and drawings were made of the changes taking place in the initial period and at convenient intervals afterwards.

::::::::::

R E S U L T S.

Tests of the Specific Gravity of Tubers.

A. Varieties of Potatoes and their Mean Specific Gravity :

The specific gravities of a number of tubers of each of 47 varieties was determined. The "range" of these varied from 1.05 to 1.13. The "Mean Specific Gravity" was calculated for each variety from the number of tubers falling in each specific gravity group. The results are indicated diagrammatically in Figure 2.

B. Effect of Manurial Treatment on the Mean Specific Gravity of Tubers :

For this test eight manurial treatments consisting of combinations of Superphosphate (P), Sulphate of Potash (K), and Sulphate of Ammonia (N) on two varieties - British Queen and Majestic - gave the following results :-

Table I./

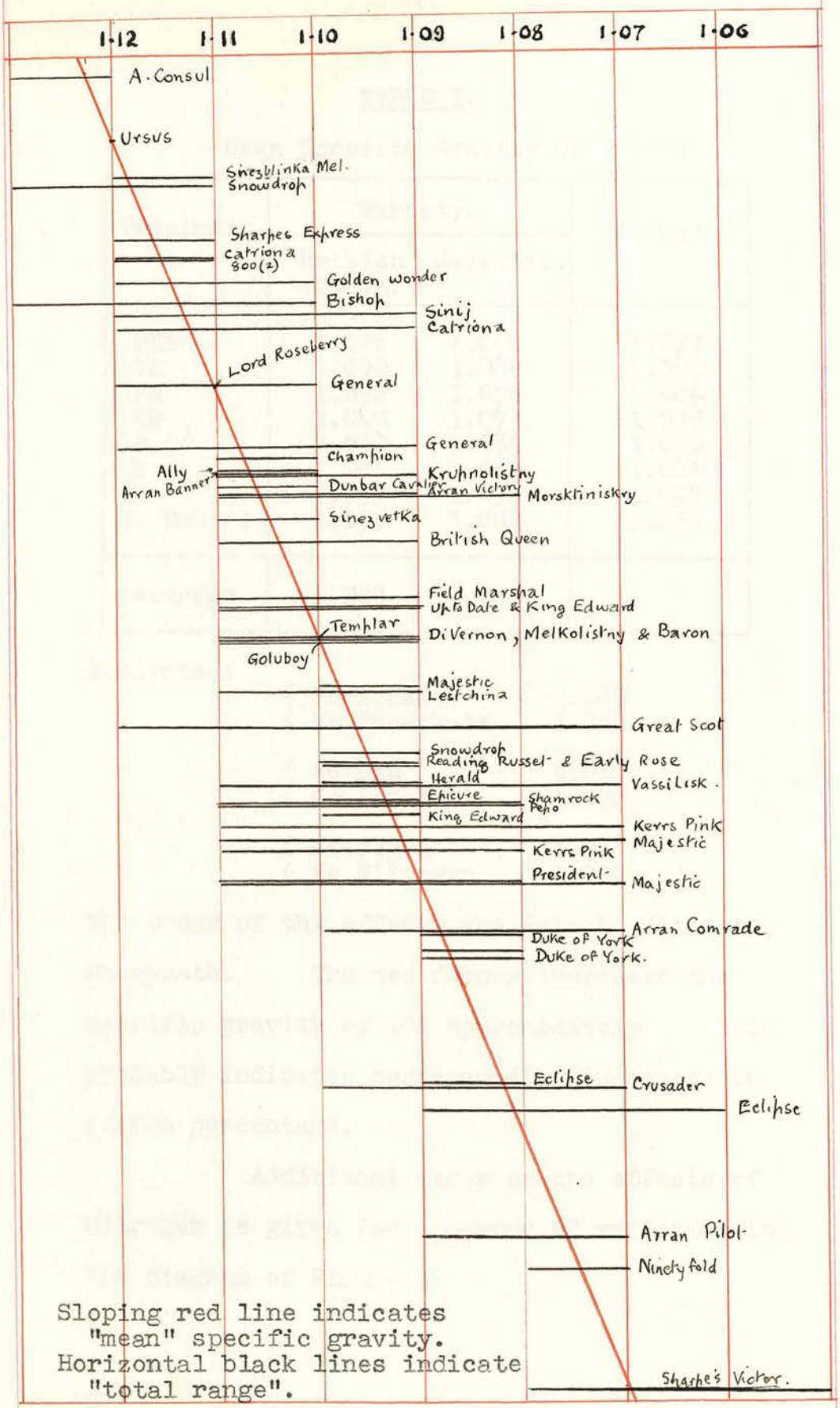


FIG. 2. Varieties and Specific Gravity.

TABLE I.

Mean Specific Gravity of Tubers.

Treatment	Variety.		Average.
	British Queen.	Majestic.	
PKN	1.076	1.078	1.077
PK	1.090	1.089	1.090
PN	1.092	1.076	1.084
KN	1.080	1.073	1.077
P	1.090	1.089	1.090
K	1.085	1.082	1.084
N	1.097	1.096	1.097
No Manure	1.104	1.092	1.098
Average	1.089	1.084	

Analysis :

(Phosphate	1.085
(No Phosphate	1.089
(Potash	1.082
(No Potash	1.092
(Nitrogen	1.084
(No Nitrogen	1.090

The order of the effects was Potash, Nitrogen, Phosphate. The two former increased the specific gravity by .01 approximately. This probably indicates corresponding increases in starch percentage.

Additional tests of the effects of Nitrogen is given for a number of varieties in the diagram of Figure 3.

C./

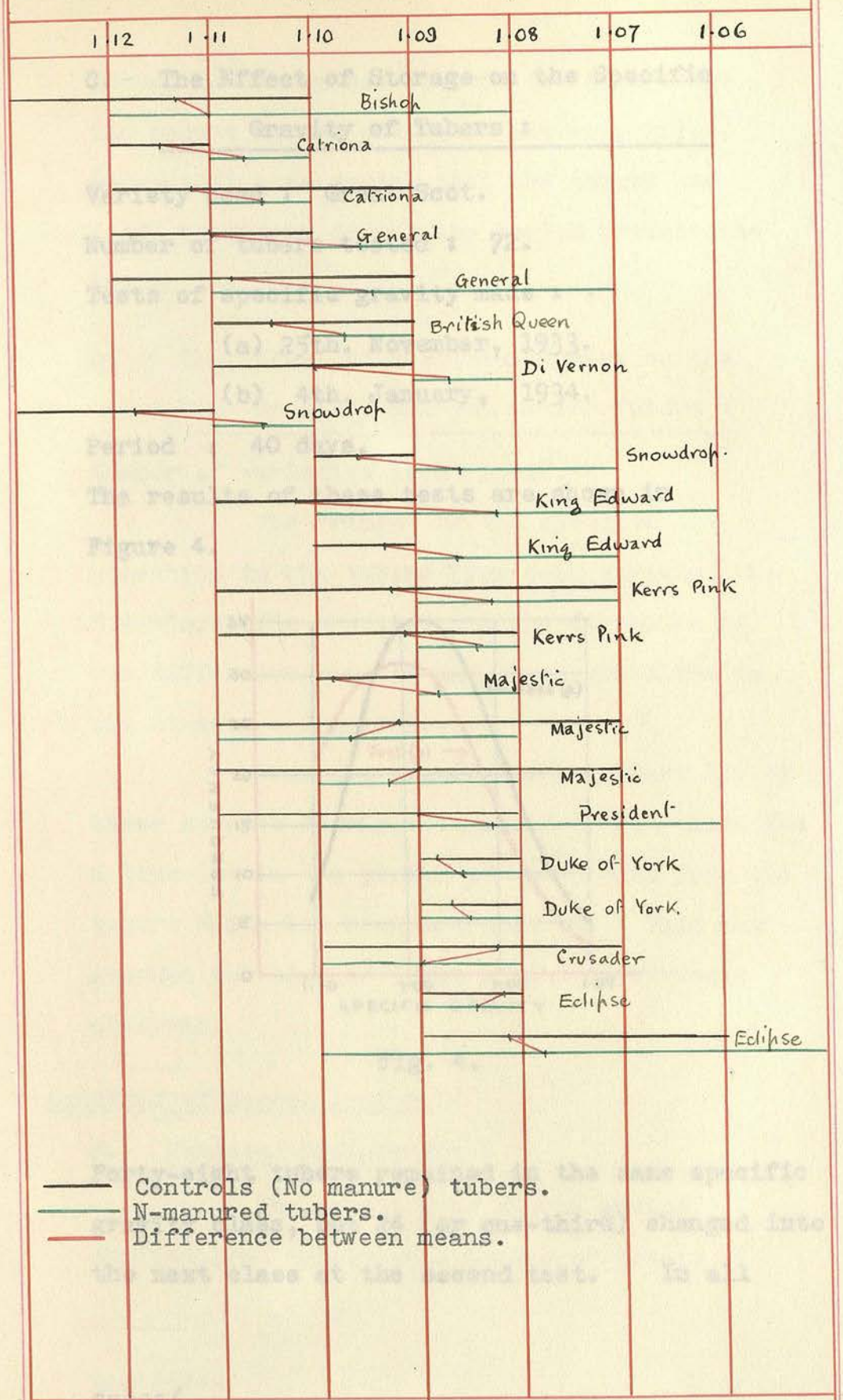


FIG. 3. N-manuring of Potato Tubers & Specific Gravity.

C. The Effect of Storage on the Specific Gravity of Tubers :

Variety used : Great Scot.

Number of tubers tested : 72.

Tests of specific gravity made :

(a) 25th. November, 1933.

(b) 4th. January, 1934.

Period : 40 days.

The results of these tests are shown in Figure 4.

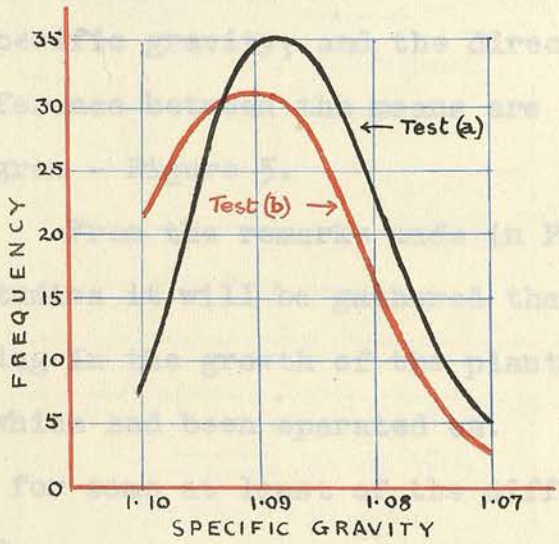


Fig. 4.

Forty-eight tubers remained in the same specific gravity class, but 24 (or one-third) changed into the next class at the second test. In all

cases/

cases the change was towards the more dense. The amount of the change was not very great, especially considering that the tubers had begun to sprout during the period between the tests.

D. The Effect of Operational Work on the Specific Gravity of the Tubers :

Number of varieties used : 28.

The "range" of the specific gravities in the tubers from each variety, the "mean"specific gravity, and the direction of the difference between the means are shown in the diagram - Figure 5.

From the remarks made in Part III of these studies it will be gathered that there was a time-lag in the growth of the plants from the tubers which had been operated on. This may account for some at least of the differences observed.

Swelling of Starch Grains.

A. Varietal Differences :

The swelling of starch grains derived from tubers of a number of potato

varieties/

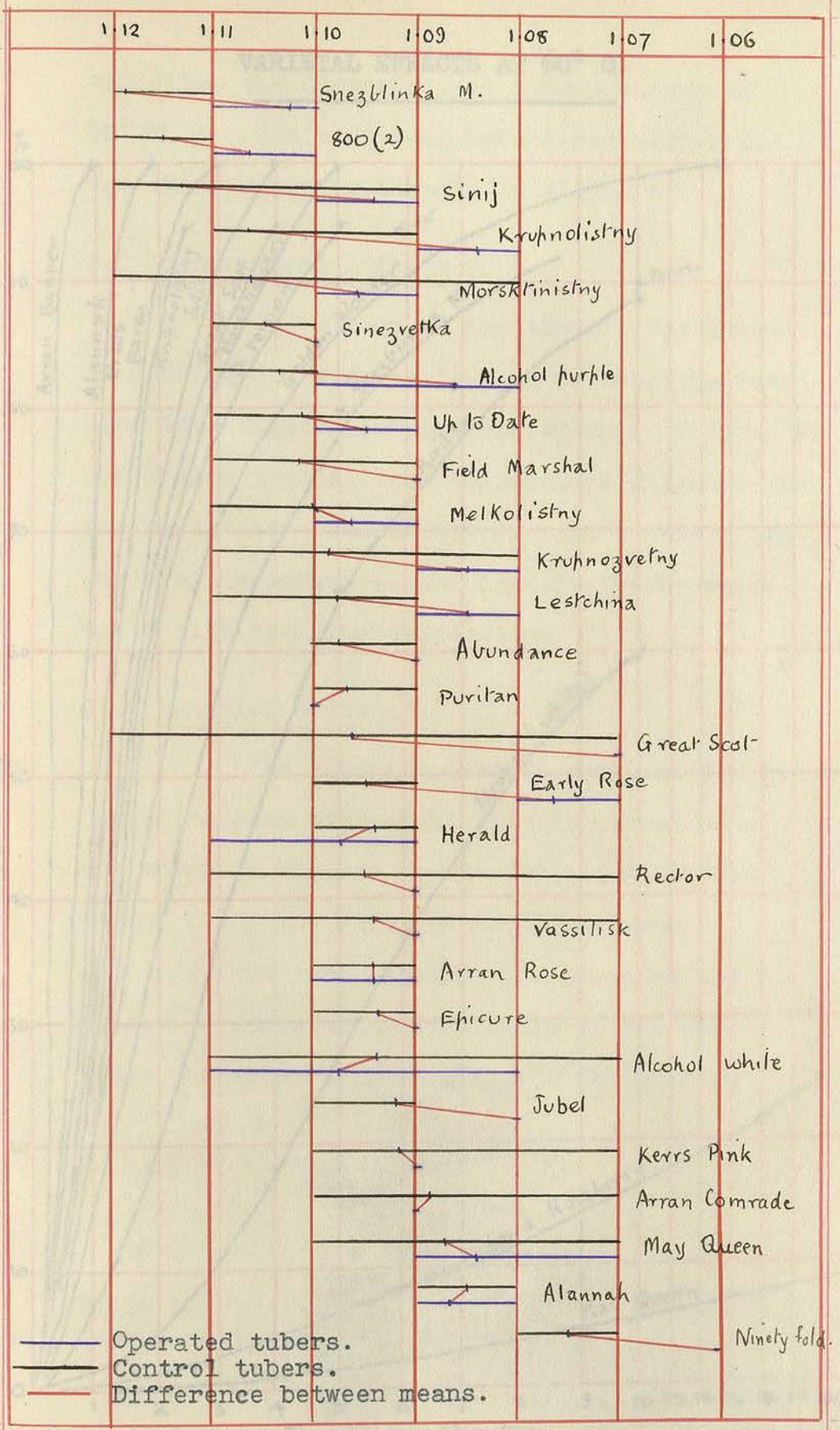


FIG. 5. Operational Tubers & Specific Gravity.

VARIETAL EFFECTS AT 60° C.

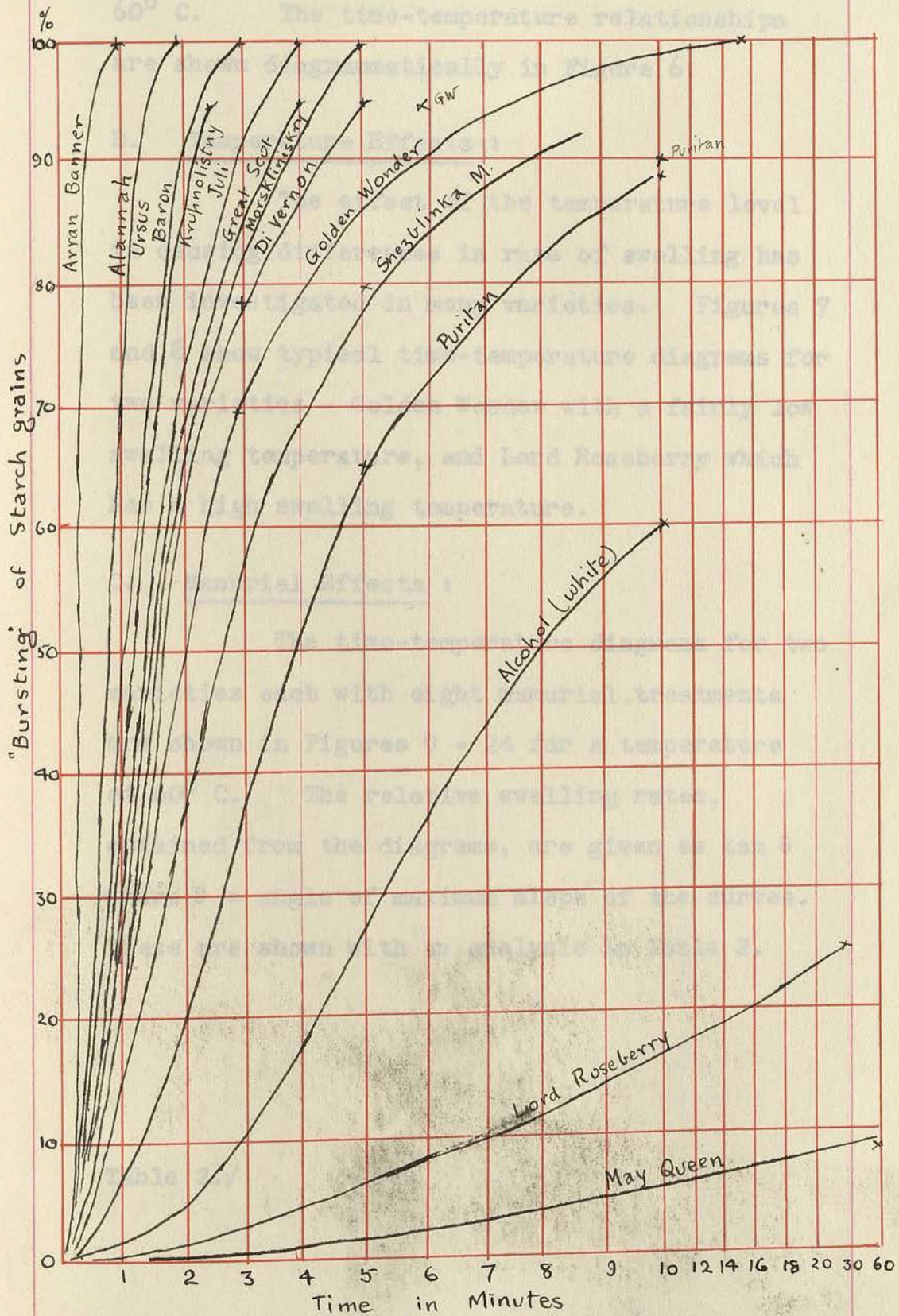


FIG. 6.

varieties was determined at a temperature of 60° C. The time-temperature relationships are shown diagrammatically in Figure 6.

B. Temperature Effects :

The effect of the temperature level in causing differences in rate of swelling has been investigated in many varieties. Figures 7 and 8 show typical time-temperature diagrams for two varieties - Golden Wonder with a fairly low swelling temperature, and Lord Roseberry which has a high swelling temperature.

C. Manurial Effects :

The time-temperature diagrams for two varieties each with eight manurial treatments are shown in Figures 9 - 24 for a temperature of 60° C. The relative swelling rates, obtained from the diagrams, are given as tan θ where θ = angle of maximum slope of the curves. These are shown with an analysis in Table 2.

Table 2./

TEMPERATURE EFFECTS.

Golden Wonder.

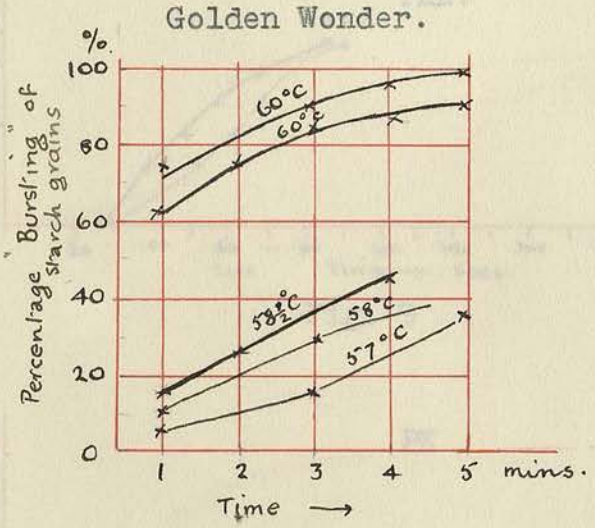


FIG. 7.

Lord Roseberry.

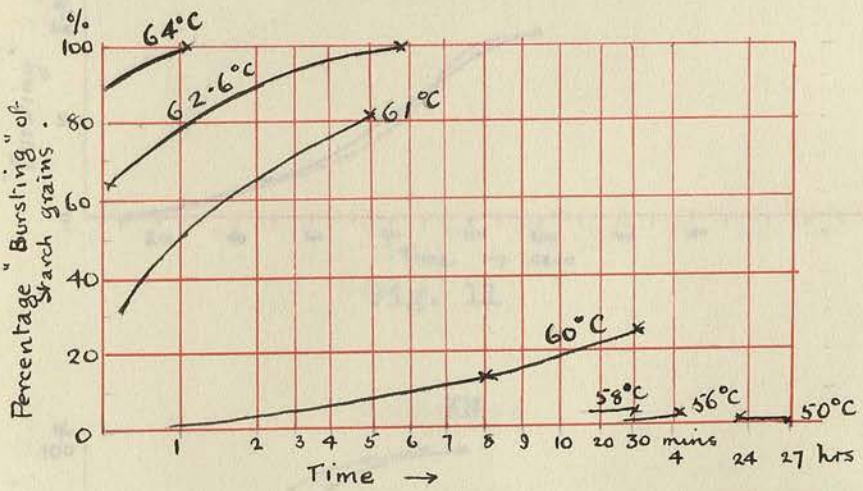


FIG. 8.

MANURIAL EFFECTS.

Variety - British Queen.

Temperature 60°C.

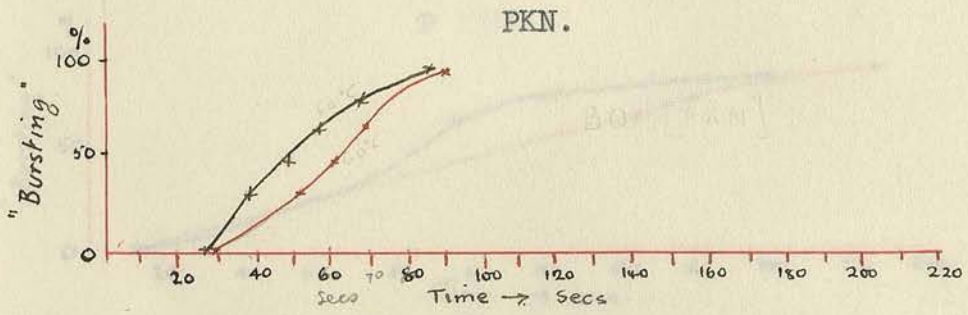


Fig. 9

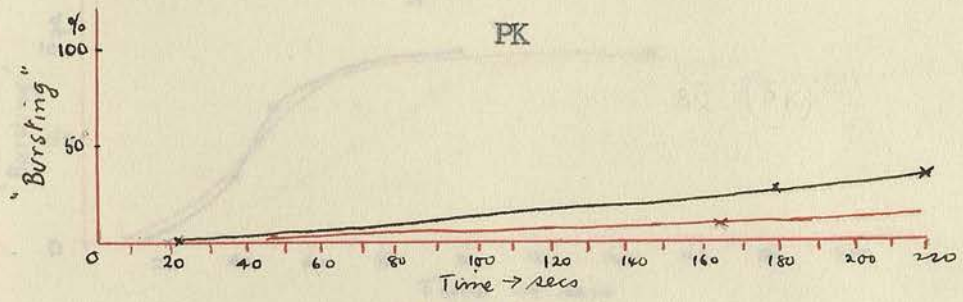


Fig. 10

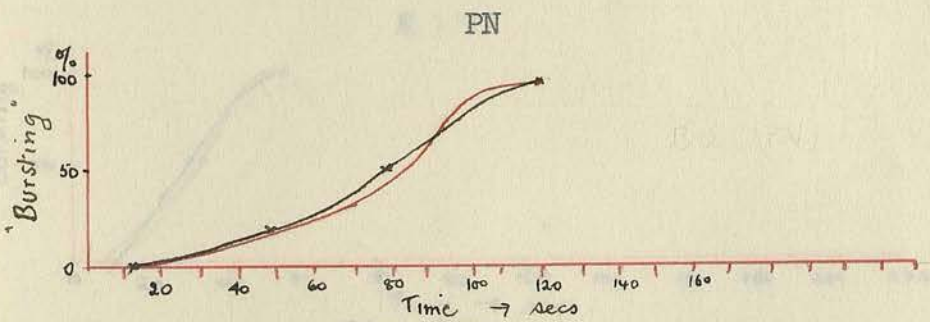


Fig. 11

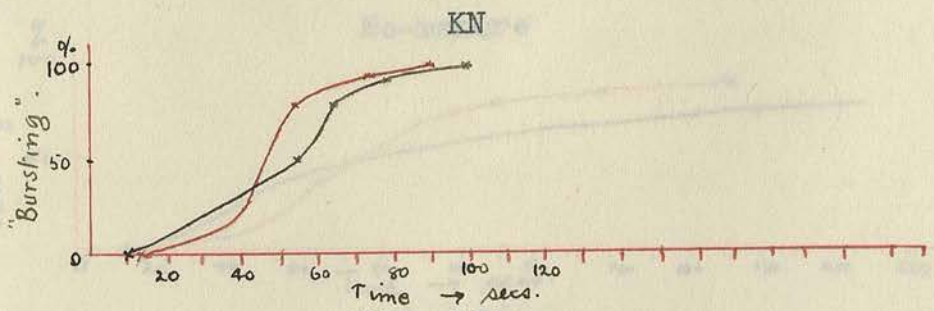


Fig. 12

British Queen - Continued.

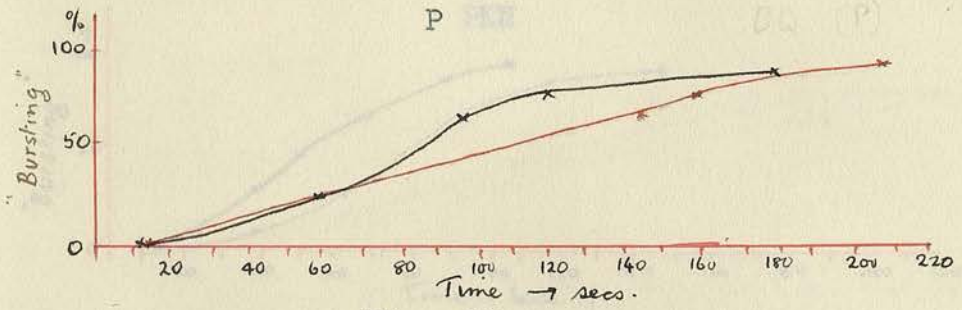


Fig. 13

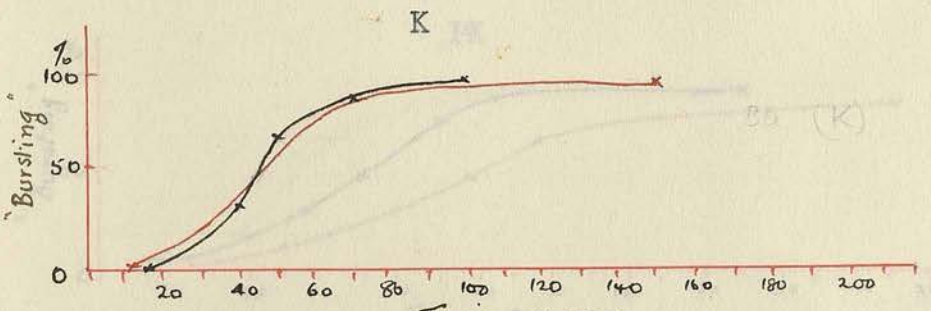


Fig. 14

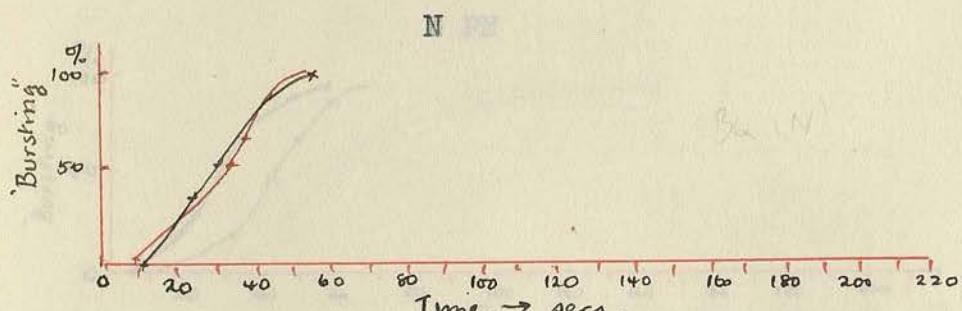


Fig. 15

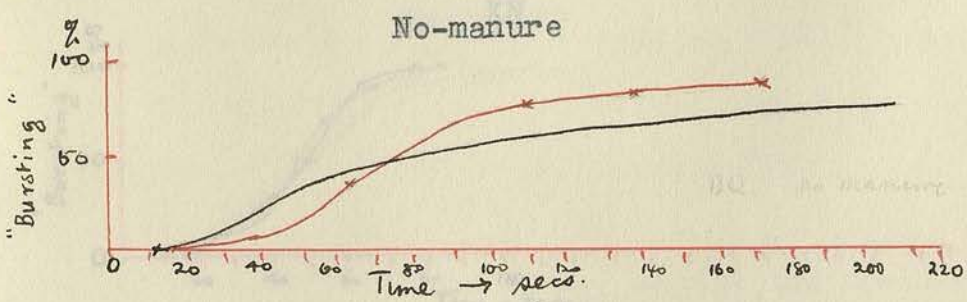


Fig. 16

MANURIAL EFFECTS.

Variety - Majestic.

Temperature 60°C.

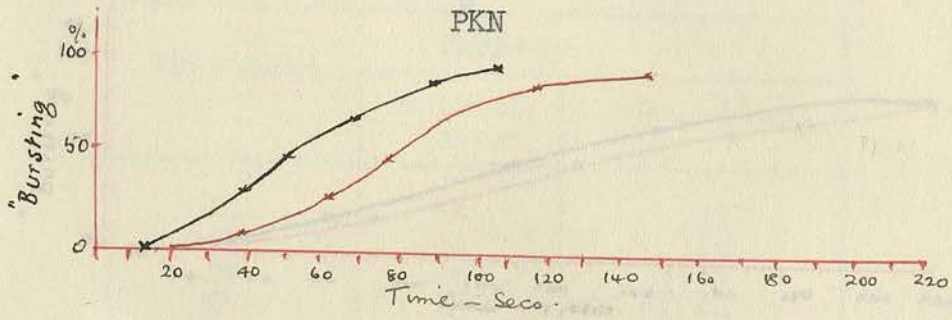


Fig. 17

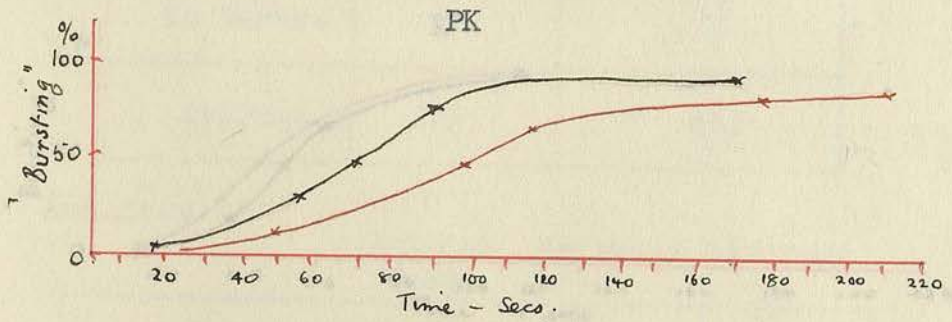


Fig. 18

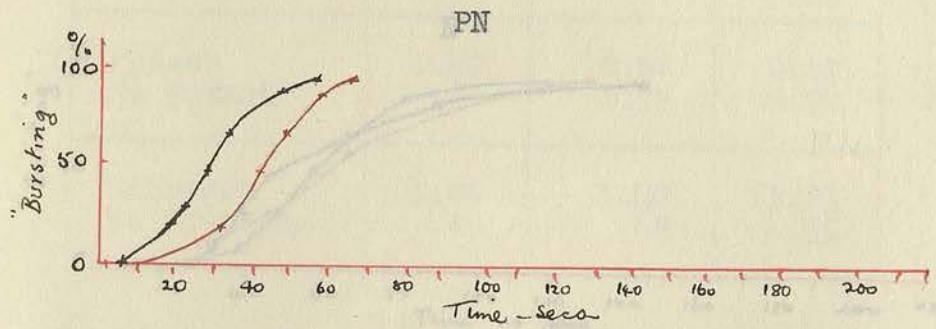


Fig. 19

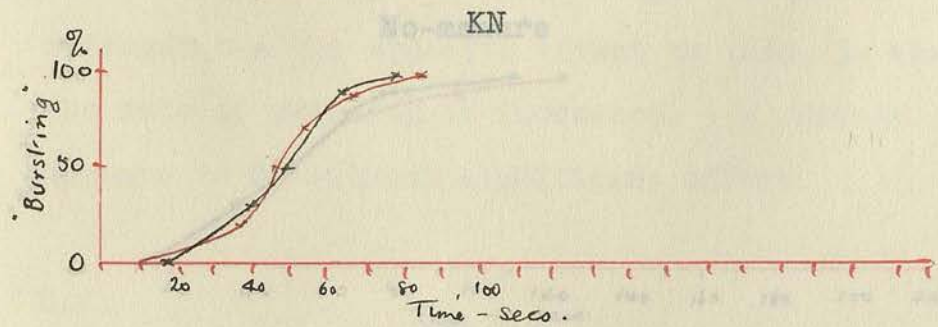


Fig. 20

MANURIAL EFFECTS.

Majestic - Continued.

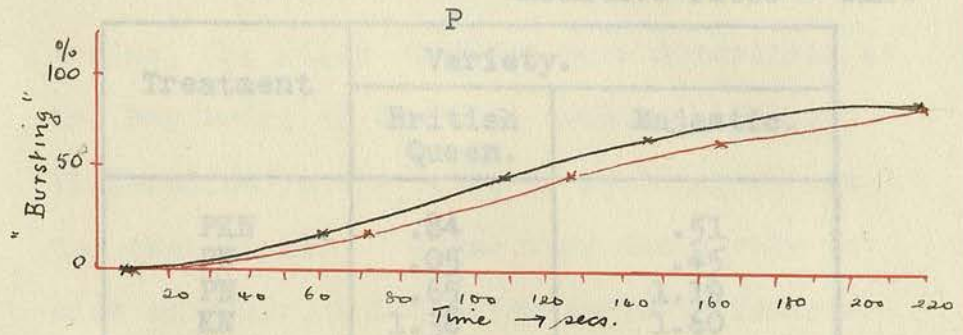


Fig. 21

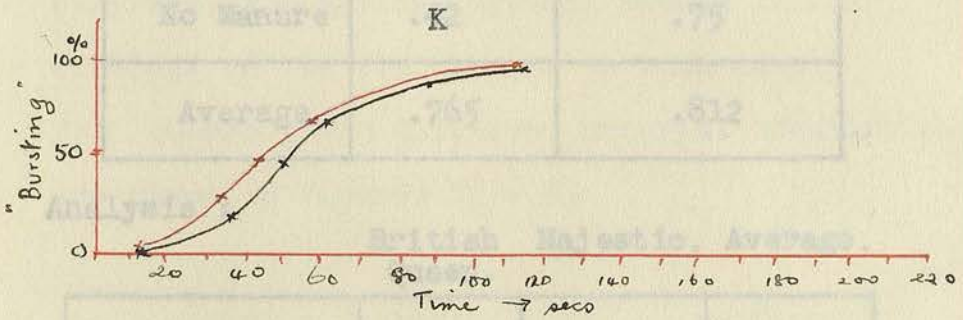


Fig. 22

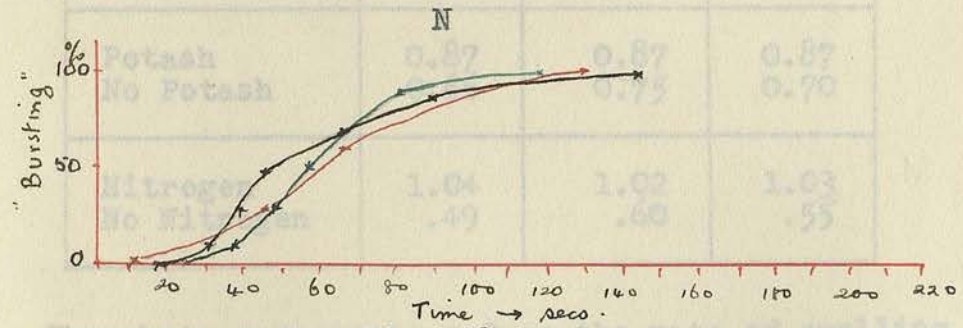


Fig. 23

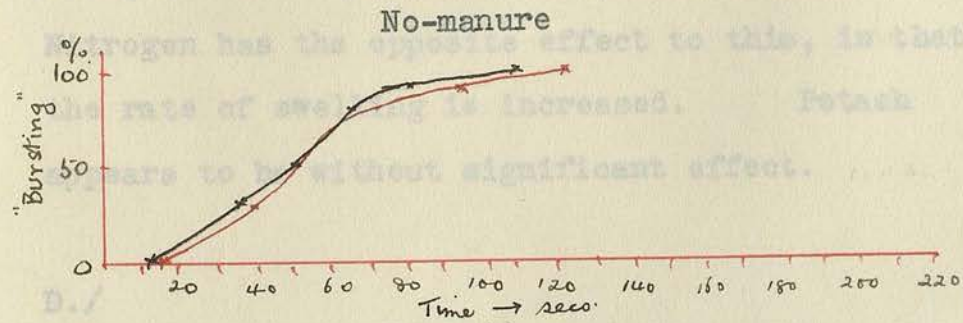


Fig. 24

TABLE 2.

Relative rates = Tan θ

Treatment	Variety.	
	British Queen.	Majestic.
PKN	.84	.51
PK	.05	.45
PN	.65	1.19
KN	1.38	1.60
P	.27	.29
K	1.23	.93
N	1.28	.78
No Manure	.42	.75
Average	.765	.812

Analysis :

	British Queen.	Majestic.	Average.
Phosphate	0.45	0.61	0.53
No Phosphate	1.08	1.02	1.05
Potash	0.87	0.87	0.87
No Potash	0.66	0.75	0.70
Nitrogen	1.04	1.02	1.03
No Nitrogen	.49	.60	.55

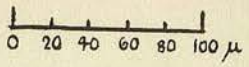
Phosphate appears to reduce the rate of swelling, i.e., the starch grains take longer to burst. Nitrogen has the opposite effect to this, in that the rate of swelling is increased. Potash appears to be without significant effect.

D./

D. Effect of Size of Starch Grains :

Observations made on four starch grains, the sizes of which were determined at the beginning of the heat treatment, showed differences in the rates of swelling within the one sample. These were due partly to size of grain, but unaccountable variations occurred in the rates for the same size grains. Diagrams showing some typical camera lucida drawings of the four grains in each sample from tubers given different manurial treatments are given in Figures 25 - 27.

::::::::::



VARIETY :
Di Vernon
TEMPERATURE :
60·4°C

Black outline = original size of starch grain.

Red outline = size of swollen grain at 5 mins in hot water (60·4°C)

Manurial Treatment

(PKN)

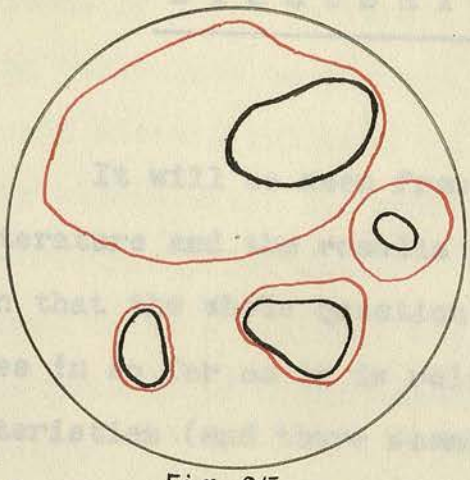


Fig 25

(K)

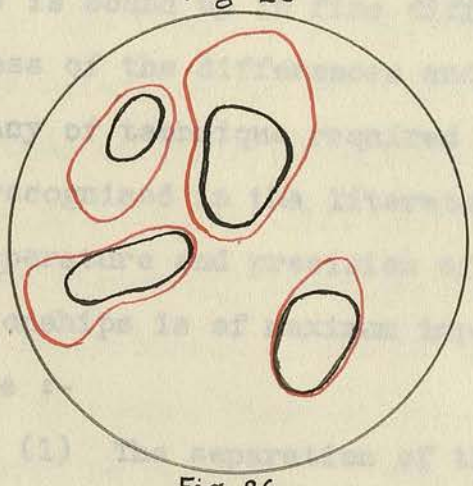


Fig 26

(N)

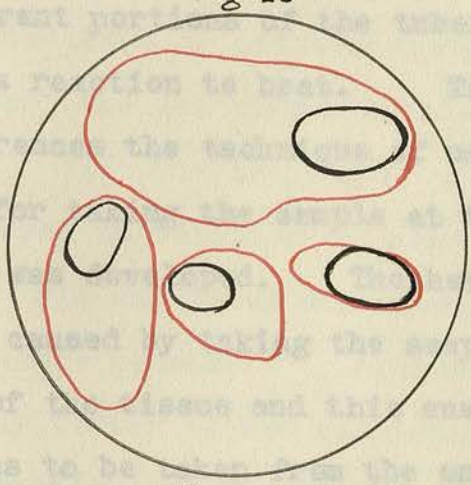


Fig 27

D I S C U S S I O N.

It will be seen from the reviews of the literature and the results obtained in this section that the whole question of quality in potatoes in so far as it is related to physical characteristics (and there seems no doubt that it is) is bound up in fine differences. The fineness of the differences and, therefore, the accuracy of technique required has not so far been recognised in the literature. Precision of temperature and precision of time-temperature relationships is of maximum importance. For example :-

(1) The separation of the starch from different portions of the tuber shows differences in its reaction to heat. To obviate these differences the technique of using a very fine tube for taking the sample at the desired tissue depth was developed. The healing of the small wound caused by taking the sample did not affect much of the tissue and this enabled a number of samples to be taken from the one tuber, allowing a survey of the whole tissue and giving accurate

comparisons/

comparisons of tuber to tuber. The tuber after sampling could even be planted if desired and subsequent history followed up. The method of using starch which has not received any form of pre-treatment, for example, heating or drying, is claimed to be a distinct advance as compared with the methods of other workers quoted in the Introduction.

(2) The use of specific gravity tests for the determination of starch is not new, but the method of employing a range of salt solutions made up to known specific gravities, together with the "floating" technique, has not been seen in the literature. This method could be refined to secure finer differences, but a limit would soon be reached. The method has produced indications of differences due to manurial treatment and the effect of storage.

(3) The heating of the starch has been considered in relation to temperature and time, and to this end tests were made keeping the temperature constant. Arzichowski (1918) is the only worker to use constant temperature and his investigation was on the starch from one tuber

only/

only and he did not develop the idea of time-temperature relation. Francis & Smith (1916), using a slowly rising temperature, gave indications of a time effect. They commenced their tests at 3 to 5° below the gelatinization temperature and applied the heat for some five minutes, rather than commence from room temperature (as did previous investigators with the application of the rising temperature for correspondingly longer periods). They found that the long period gave indefinite results. This indicates that the "rate of swelling" is the significant quantity to be determined in gelatinization of starch grains. "Swelling" is regarded as synonymous with loss of anisotropic properties or the taking up of stains, for example, Congo Red, as mentioned by Huss (1922). The view of Arzichowski that the temperature should be maintained to an accuracy of 0.1° C. or better is confirmed. Fluctuations of such small magnitude as to be within .1° C., however, are shown to be not negligible for the swelling of potato starch at least about 60° C. Arzichowski found that an increase in temperature of .1°C., corresponded to an increase of over 2% in the

number/

number of completely swollen grains. The significance of this factor is not the same at all temperatures, as at lower temperatures the rate is not so high. The method used in the present investigations for the determination of the "rate of swelling" of starch grains indicates :-

(a) marked differences between varieties at 60° C.,

(b) differences due to manurial treatment of the plants, and

(c) possibilities in the determination of the effect of size of the starch grain.

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S U M M A R Y.

(1) Determinations of the swelling rate (time to gelatinization) of individual starch grains from different varieties of potato tubers are reported.

(2) Definite differences in the swelling rate between grains of different varietal origin are obtained.

(3) The relationship between these varietal differences and quality are discussed.

(4) The effect of manurial treatments of the parent plant on starch characteristics is shown and discussed.

(5) Considerable advances in technique are described.

(6) The most significant advance is in the design of an apparatus which allows of :

(a) Direct observation of individual grains under a microscope.

(b) Immediate approximation to within 0.1° C. of the desired temperature direct on to the grain.

(c) Observation of the polarization character of the grain while swelling.

(7)/

(7) The influence of pre-treatment of test samples of starch is discussed and the importance of direct immediate transferral of starch from tuber to test emphasized.

(8) Refining of the technique has shown that the time taken for the test grain to reach test temperature from room temperature affects the gelatinization time.

(9) It is shown that if the grain is raised immediately to the gelatinization temperature the time taken for gelatinization is short, but if the rise in temperature is gradual the time taken after gelatinization temperature is reached is extended.

(10) At a temperature rather below the actual gelatinization temperature the grain will swell and eventually burst given time; there is a relationship within limits between time and temperature of the nature of $T \times t^{o}$.

(11) There is every indication that there is a relationship between the specific

gravity/

gravity of the whole tuber and the gelatinization characteristics of the individual starch grains from that tuber. The specific gravity and characteristics are both influenced by manurial treatments and varietal nature.

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PART II.

STERILITY DUE TO ABSCISSION.

C O N T E N T S.

INTRODUCTION.

In plants, when the marketable product depends on effective flowering and fruiting, (e.g. Cotton) autonomous cutting off of flower buds, flowers and fruits is of major importance and has received a considerable amount of attention, much of which is germane to the present study and will be discussed later. In the potato where active breeding work is enforced by the demand for new varieties with higher quality, disease resistance, etc., the question of abscission has become of paramount importance, for the high degree of cutting off of the reproductive units practically amounts to total sterility. Also considerable wastage of effort due to treated (emasculated and hybridised) reproductive structures falling off occurs. In the potato the appearance and development of the reproductive phase seems to be conditioned by climatic factors to a great extent and these will receive considerable attention.

Garner & Allard (1920, 1923, 1931) dealt with their studies on photoperiodism and found there was an optimal light duration for tuberization as well as for flowering and fruiting and for purely vegetative development. McClelland (1928) found there were varietal differences in the response by potatoes to the duration of light.

Lyssenko (1932) disagreed with the common interpretation of photoperiodism which stressed the necessity for an alteration of light and darkness. He pointed out that flowering in the potato was inhibited entirely by short-day conditions and the potato was thus to be regarded, from the ordinary viewpoint, as a long-day plant. Long-day plants reached the stage of reproduction most rapidly of all when grown under continuous illumination, i.e. when there was no alteration whatever. Lyssenko's fundamental postulates were :-

(1) The "development" of an annual seed plant, e.g. the potato, consisted of a sequence of qualitatively different individual stages occurring in the plant or in its individual organs

without/

without which it was impossible to obtain true seed.

(2) The "growth" of a plant was understood to refer to the increase in the weight and size of a plant at any given stage of development. The growth of the plant was different at different stages of development.

(3) The processes of "development" and of "growth" were two distinct phenomena and not necessarily antagonistic one with the other. Consequently, it was possible, e.g. to subject the plant for a certain period to the influences which favour its reproductive development, during which time its growth was temporarily kept in check, and may later be transferred to other conditions favouring growth; in this way the two sets of processes were duly accelerated.

Lyssenko found that potatoes grown under continuous illumination developed quicker and, in most cases, all the products of photosynthesis were

used/

used exclusively for plant development and not for tuber formation. When the cycle of development was completed by fruit formation (frequently it did not complete this owing to the loss of flowers and flower-buds by abscission) the nature of the potato plant was such that it did not perish, but continued to assimilate and to use the products of assimilation entirely for tuber formation.

Short-day conditions prevented entirely the formation of flowers in the potato plants, i.e. there was an arrestment of the progress towards reproduction, but this retardation of development did not mean the retardation of photosynthesis or increase of the plant mass. The products of assimilation accumulated under the short-day conditions were partly or entirely used for stolon and tuber formation. Lyssenko thus regarded the tuber formation by the potato plants exposed to short-day illumination as actually due to the retardation or possibly to the absence of plant development. If experimental plants of potatoes be transferred from short to long-day conditions, the plants ceased to form tubers and proceed

towards/

towards flowering. If conditions be favourable for accelerated development, then the products of photosynthesis accumulated by the plants at that time could be entirely used up while the deposits in the tubers could also be drawn upon. In Lyssenko's trials with potatoes at Odessa, the plants under continuous light formed flowers and then commenced tuber formation which began earlier than in the plants of the same variety grown under normal conditions. The plants grown under a shortened day of 9 or 12 hours had a stocky aspect with shortened stem internodes and did not flower. The yields per plant in the different treatments were :-

Under continuous illumination - 374 grams
Under normal day illumination - 260 "
Under 9-hour day illumination - 250 "

Lyssenko concluded that in order to accelerate tuber formation of a potato plant under field conditions, neither preparatory development of the plant nor flowering must be hindered, and in essence the technique for vernalizing potatoes was the application of continuous light for a few weeks before planting in the field.

Stevenson/

Stevenson & Clark (1933) found that by increasing the period of exposure to light by 6 hours daily for 60 days, the plant growth and flowering were stimulated to a remarkable degree, but tuber production scarcely at all. There was a high correlation between the capacity for setting seed by natural fertilisation in the field and the number of flowers produced in the glass-house under lights. It will be seen from the above literature that the period of exposure to light affects flowering in the potato, but it is to be regretted that no figures are available for the number of flowers produced or the dropping of flowers, flower-buds and fruits. However, there is every indication of varietal responses.

While no information is available on the effect of abscission by light, there is a considerable literature on the effects of temperature, humidity and nutrition. Kendall (1918) found that both the reaction time and the actual abscission time were profoundly influenced by temperature and by humidity, being far more dependent on temperature than on humidity. Abscission went on very actively under high

temperature/

temperatures and conversely very slowly under low temperature. For example, in Tobacco hybrids treated with 1½% illuminating gas, abscission started in about 7 hours at 19° C., but it might not occur for 15 to 24 hours at 9° C. Dorsey (1919) stated that cooler conditions were generally held to be favourable to the development of "seed balls" in the potato. Young (1922) noted in his studies of abscission in the potato that moderately cool weather, especially at night, favoured the setting of seed, and that a gradually falling temperature with a moderate amount of moisture was especially favourable. It was not unusual for a wave of warm weather in early summer to be followed by the nearly or quite complete shedding of the buds and blossoms of the potato. Stout & Clark (1924) stated that the first condition necessary for the successful breeding of the potato was that of certain and profuse blooming. They found that nearly, if not all, varieties bloomed in profuseness in the region about Presque Isle, Maine, (46½° North), where the summers were cool and the growing season only about 100 days, but seasonal variation was evident.

Arthur/

Arthur & Guthrie (1927) studied the effect of light, CO_2, and temperature on flower and fruit production. They found that potatoes grown at 26° C. produced very weak stems, few flowers, and only small tubers, even with additional CO_2 and light, while those grown at 20° C. produced strong stems, large tubers and flowered especially well in a green-house with additional light and gas. This showed that the potato is definitely limited to a low temperature and was not able to utilise additional light and CO_2 at high temperatures.
Smith (1932) working with tomatoes found a lag of approximately 3 days between the time the temperature exerted an effect on anthesis and blossom drop and the time that the effect became visible. This statement could not be checked up from the data given in his paper.
Semsroth (1934) showed that for a good setting of berries in artificial pollination of potato flowers, the daily average temperature should be low (18.7° C.), a low average maximum (22.5° C.) and the humidity high (79%). A bad set resulted when the daily average temperature was 21° C. a very low minimum and a high maximum and a low

relative/

relative humidity. Heavy showers on the days of emasculation and pollination also resulted in a bad set. Of all the factors Semsroth regarded humidity as perhaps the most important.

Kendall (1918) stated that drought had to be quite severe before retarding abscission. He found that wilted shoots would not drop flowers as quickly as fresh ones, and if the wilting proceeded far enough no abscission would occur. This effect was all the more noticeable if the air around the wilted shoot was kept free from moisture. Smith (1932) found that hot dry winds have little effect on the blossom drop in the tomato. It was noticed that the dropping was most severe when the soil moisture was dificient.

Kraus & Kraybill (1918) found that nitrates played a very important part in the development of the abscission layer. High carbohydrate content made for continued development of the vascular strands of the pedicel and the strengthening of their connection (c.f. greater thickening of the xylem cells in other parts of plants when nitrates were used), yet marked abscission occurred when carbohydrate content was

very/

very high. The greatest number of flowers in the tomato were produced neither by conditions favouring highest vegetation nor by conditions markedly suppressing vegetation. Kraybill (1926) showed that the number of blossoms which dropped was influenced by the amount of available mineral nutrients under conditions of uniformity with respect to other external factors. Smith (1932) found that heavy fertilisation with nitrate of soda, sulphate of potash and acidulated rock phosphate failed to reduce the amount of blossom drop. Taylor (1932) observed that applications of nitrogenous fertiliser, (cotton seed meal, and urea) in the autumn and early winter increased the number of flower clusters, flowers and fruits in strawberries. Nitrogen applied late in the growing season immediately preceding ripening tended to suppress flower and fruit production. Laurie & Poesch (1932) recorded that heavy nitrogenous fertilisation with sulphate of ammonia did not retard flower formation in chrysanthemums.

It has been found that abscission of flowers can be partly prevented by certain mechanical means. Knight (1806) reported that

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the removal of tubers during growth reacted on the potato plant by causing a greater formation of flowers. Broili (1919) found that girdling or ringing the potato stalks by wrapping wire several times round the stems at a point about three or four leaves below the top in such a manner as to inhibit the flow of sap, produced a great increase in the number of seed balls. There was no mention of any increase in number of buds or flowers, so presumably this meant that there was less abscission. Bornemann (1920) obtained similar results by "gassing" the plants with CO_2. Black (1932) found that the most successful of several methods for inducing a greater number of flowers was the simple process of bending the stem of the potato plant through 180°.

To summarize for practical purposes the methods available for reducing abscission of flower buds in the potato and thus ensuring an increase in number of seed balls are :-

(1) Girdling or ringing the potato stalks by wrapping wire round them.

(2) Bending the stem through an angle of 180°.

(3) Removal of tubers when formed.

(4)/

(4) Artificial pollination with viable pollen.

(5) Exposure to continuous light.

(6) Providing cool temperature.

(7) Increasing the food supply.

(8) Gassing with CO_2.

In organization the inflorescence of the potato is described as a monochasial cyme. The main peduncle, or flower-stalk- though lateral, occupies a central position having become stronger in its development than the stem tip, pushing aside the latter which comes to occupy a position apparently lateral. The disposition and number of the flowers and buds in the cyme has not received any attention in the literature, but in the present studies results are reported bearing on this subject.

Salaman (1926) divided inflorescences in the potato into two types, namely :-

(1) "Simple", when the flower-stalk divided once into several, usually two, secondary axes each of which formed a scafoid cyme.

(2) "Compound", where the two or more secondary axes of the simple type

again/

again divided, forming tertiary axes and, on a further division, axes of the fourth or fifth in order result and in their turn ended as a scafoid cyme.

In the descriptions given for about 80 varieties, Salaman indicated only one with a compound inforescence and one variety having compound occasionally. The remainder of the varieties, some 78, were classified as having simple inflorescences. He omitted entirely to mention types in which the flower stalk did not divide at all and it appears more reasonable to the present writer to classify inflorescences into n + 1 types, where n is the order of subdivision of the main flower stalk.

The flowers of the potato are pentamerous and are borne on bractless pedicels. They are homogeneous and produce no nectar, so are rarely visited by insects which effect pollination. Normally, they are self-pollinated and, in many varieties, the flowers do not open at all but soon wither and drop off. The lengths of the pedicels vary according to the position of the flower on the cyme, the variety, and the manurial

treatment as well as other environmental factors and diseases, such as mosaic. The pedicels show the histological features characteristic of the stem, but the vascular tissue forms a more or less continuous band instead of being arranged in distinct groups.

The finding of reference points to describe the position of the separation-layer in the potato presents considerable difficulty. Kendall (1918) described the separation-layer as being located near the middle of the pedicel and suggested that if one considered the pedicel to be composed of two internodes the layer occurs at the base of the more distal. Dorsey (1919) in investigations of the dropping of flowers in the variety "Early Ohio" used the rather indefinite flower as his reference point and noted that the joint in the pedicel at which the flowers dropped was 3 to 5 mm below the flower.

Abscission has been defined by several writers. Kendall (1918) observed that abscission in potato flowers resembled that in the tomato and the separation-layer in both cases was preformed ready to function at any stage in the development of the flower. It represented a

portion/

portion of the primary meristem which had retained some of its originally active condition and physiological capacities. The separation cells were characterised by their small size (not related necessarily to abscission), equal diametric shape, large amount of protoplasm and somewhat collenchymatous appearance. Various tests with stains, acids and alkalis failed to indicate any chemical difference between the cell walls of the separation cells and the walls of neighbouring cortical cells which do not separate. He made it clear that in most of the berry-forming species of Solanaceae the mechanical tissue of the pedicel does not become continuous through the separation-layer and thus no impediment to flower drop was afforded when abscission occurred in that region. He described the abscission process as conforming to the usual type which involved the separation of cells along the plane of the middle lamella of the cell wall separating them. No cell divisions or elongations were observed to accompany abscission. All the cells across the pedicel in the region of the separation-layer took part in separation except the tracheae and cuticle

which/

which had to be broken mechanically. The total number of cells which might be involved was greater in some species than in others and the number varied in the same species because of changes in the external conditions. Cell separation was brought about by the hydrolysis and consequent dissolution of the middle lamella, the agent active in the hydrolysis probably being enzymatic. An increase in cell turgor frequently occurred during abscission, but probably served merely to hasten and facilitate the process. Most of the frequently observed expansion and the turgid appearance of the separation cells during abscission were probably due to the natural release of pressure caused by the dissolution of the middle lamellae.

He further distinguished between "reaction time" (due to the stimulus, e.g. narcotic vapours) and "abscission time" or the actual time involved in the process of cell separation. The age of the flower was the most important factor in determining the reaction time, older flowers nearly always responding more slowly to stimulation than younger ones. The temperature had the most important conditioning effect in

estimates/

estimates of time of abscission. Goodspeed & Kendall (1916) had previously noted that the "abscission time" in "spontaneous" abscission (i.e. due to the stimulus) could be more exactly determined than in "normal" abscission (i.e. due to absence of stimulus).

Namikawa (1926) defined abscission as "the amputation of an organ by means of the isolation of living cells in a special separation-layer". Lloyd (1927) found two types of abscission :-

(1) Separation of cells <u>in situ</u> by chemical alteration of the middle lamella and adjacent secondary walls.

(2) Separation of cells produced by renewed meristematic activity followed by chemical changes in the walls.

The various conditions known or supposed to lead to abscission of the buds, flowers and fruit in the field were set forth, namely :-

(1) The destruction of pollen by rain.

(2) Failure of anthers to open during low temperature.

(3) Injury.

(4) Water stress, procured both directly and by the lack of photosynthates due to insufficient light.

(5) Mechanical strains due to disharmony of growth between the pedicel and stem.

Dutt (1928) found that the abscission process in cotton was one of physiological changes within the cell content and walls, involving the disappearance of starch and Calcium, and cellulose became hydrocellulose. Before abscission was initiated the cells of the separation zone were well supplied with starch which was much in excess of the amount present in the tissues on either side. This relationship was reversed as soon as abscission began.

The effects of manurial ingredients on the growth of plants have received much attention. The increased leaf and stem growth, the darker green foliage and the retardation of ripening are characteristic visible features of potato plants receiving nitrogenous manures, but Scott (1932) has shown that each 1 cwt. of Sulphate of Ammonia (up to 3 cwts. at least) can be expected to produce an increase of 1 ton per acre in the yield of tubers. The visible effects of phosphate manures are not obvious in the top growth, but it is well known that this constituent

affects/

affects root development to a significant extent. Increased yields of tubers resulting from applications of phosphates have been reported from many countries, especially conspicuous responses being in places where the soils were low in phosphate content. Potash, on the other hand, does not give such marked increases in yield, but Russell (1932) pointed out that the efficiency of the leaf as a producer of carbohydrate might be raised by increasing the supply of Potash. The usual effects of these manurial ingredients were observed in the work herein described. Attention has been particularly directed, however, to the effects of Phosphate, Potash and Nitrogenous manures (alone and in combination) on the inflorescences of potato plants.

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MATERIAL and METHODS.

Healthy seed tubers of a number of varieties were obtained through the courtesy of the Directors of the Scottish Seed Testing Station and the Scottish Society for Research in Plant Breeding, to whom thanks are due for assistance in this work. These tubers were carefully marked with indelible pencil immediately on receipt and treated with acidulated Mercuric Chloride for the control of surface borne fungus diseases before being given the "greening" process by exposure to light.

For the trials undertaken a sample of each variety as uniform as could be obtained was divided into eight lots and planted at the proper time into a free working garden loam in 10" pots. The pots were placed so that they were well isolated from any other Solanaceous plants and arranged that every pot had comparable conditions of light, etc. At planting time manures were applied at a rate corresponding to a field dressing of approximately 5 cwt. per acre of each of the three main manures in various

combinations/

combinations. In arriving at the quantity to be applied it was thought advisable to calculate on the basis of the number of plants per acre rather than from the actual surface area of the pot which gives very much smaller area for each plant than occurs under the field conditions. The quantity of any single manurial ingredient applied was one-third ounce per pot. A second application of manures was made when the plants were about 1 foot high.

Frequent watering was resorted to so that at no time was the soil allowed to become dry. The day following watering the surface soil of the pots was stirred. In the 1933 crop the pots were allowed to rest on top of the ground and when some of them were moved it was noticed that roots had penetrated through the drainage hole of the pot into the ground below. These roots were broken in the process. In the 1934 planting, the pots were partly plunged in soil and were not moved until harvest. This prevented breaking of roots and further assisted in maintaining the moisture supply.

The sets were planted about 2" in the soil. In 1933, planting was done on 29th. May,

and/

and in 1934 on the 5th. May. The varieties used in abscission work were :-

1933.	Arran Consul	Eclipse
	Bishop	King Edward
	British Queen	Kerr's Pink
	Catriona	Majestic
	Crusader	President
	Di Vernon	Sharpe's Express
	Duke of York	Snowdrop
		Templar.
1934.	Ally	Golden Wonder
	British Queen	Great Scot
	Catriona	King Edward
	Di Vernon	Majestic
	Duke of York	President
	Epicure	Up-to-Date

In 1933 the number of varieties given complete sets of eight manurial ingredients was two owing to scarcity of material, but in 1934 the work was extended so that 12 varieties were given all the treatments. Observations on the emergence of the bud clusters, opening of flowers, formation of fruits, dropping of buds, flowers and fruits, etc., were made daily (with very few breaks) on all plants until the haulms had withered away. The various combinations of Superphosphate (P), Sulphate of Potash (K), and Sulphate of Ammonia (N), were : PKN, PK, PN, KN, P, K, N, No Manure. These made the series of 8 treatments for each variety with two levels of each manurial constituent.

An/

An attempt to alter the environment , particularly the moisture conditions, consisted of placing very large glass bell jars over a number of plants and bedding down the jars on the top of the pots which had been covered with a layer of soft wax mixture composed of Paraffin Wax (M.P. 54° C.) 80%, Vaseline 20%. The roots of some of the plants were given water continually through the soil, while others were kept comparatively dry. Where moisture was required both at root and shoot, the wax layer was omitted. A drying substance, anhydrous Calcium Chloride, was employed in an attempt to keep the atmosphere dry round some of the plants. The transpiration was so great, however, that the desiccating agent was not effective. It was also found that the bell jars reduced the light somewhat and made the plants grow so tall that the foliage congregated in the tops of the jars. In the following year four plants of each of three varieties were given reduced period of exposure to light by putting them in a dark room except for nine hours, namely, from 9 a.m. to 6 p.m., during which period they were in the light. The 12 control plants were given the ordinary daylight period. The

results/

results from the two series cannot be regarded as comparable because the whole environment of the plants in the dark room was altered compared with that of the controls.

In the first season the records were taken daily (except when stormy weather prevailed), from 16th. July to 4th. October, (when cold weather killed the remaining plants), a period of 80 days. At first it was decided to allow the flower-buds, flowers, etc., drop of their own accord, but observations showed that in many clusters complete abscission had occurred and the buds were just "sitting", or were held in by the leaves and other buds. Hence it was decided to touch each bud very lightly. The change in the records occurred within a couple of days when the first buds were cut off and affected the time of drop, but not the number, of these few buds. In 1934 the records were made more complete by early counts of the total buds present and by taking daily counts (in wet or fine weather) from 24th. June to 7th. November, a period of 136 days. Very few buds dropped after 4th, October, when the first spell of cold weather killed the plants and caused the tops to

wither.

The weather records for the periods of observation in both seasons are shown graphically in Figures 28, 29, and give the following readings :-

(1) Maximum and minimum temperatures.

(2) Mean daily temperatures.

(3) Humidity.

(4) Rainfall.

(5) Wind and cloud.

The large mass of data accumulated has been partially analysed statistically by the methods given by Fisher (1932), Yule (1932), Tippett (1931) and Banister (1929). Since many of the classes are incomplete owing to accidents to plants, analyses were made in sections which were complete. The initial figures, (Tables 3, 4, & 5), Analyses of Variance, Summaries, and Tests for Significance by the Methods of S.E. and "Z" are shown for the calculations of variation within and between classes. For correlation tables, however, the correlation coefficients and their significance only are given. The original data concerning the number of flower-buds, flowers and fruits formed,

1934

WEATHER RECORD.

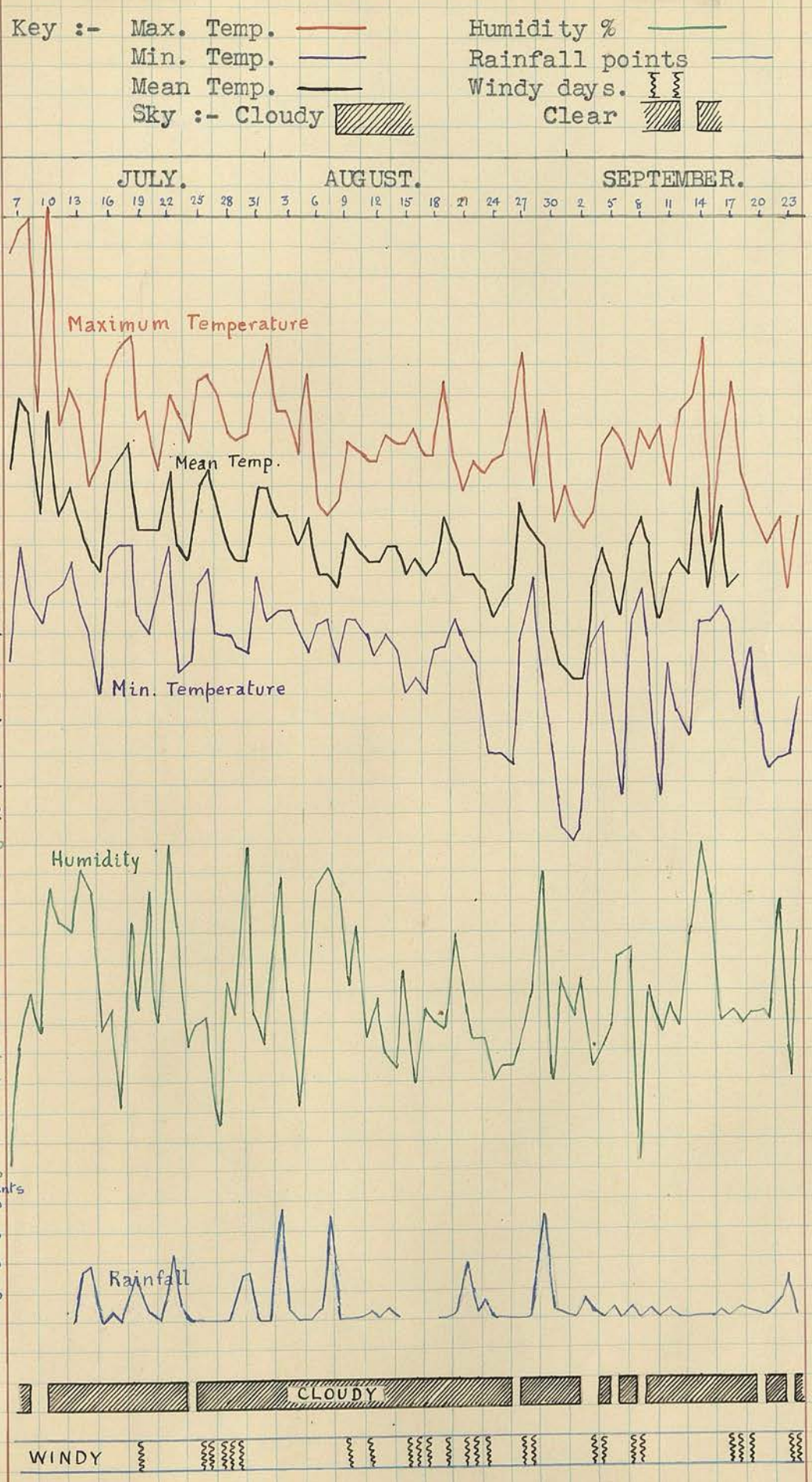


Fig 28

WEATHER RECORD
1933

Key: Maximum Temperature
Minimum Temperature
Humidity

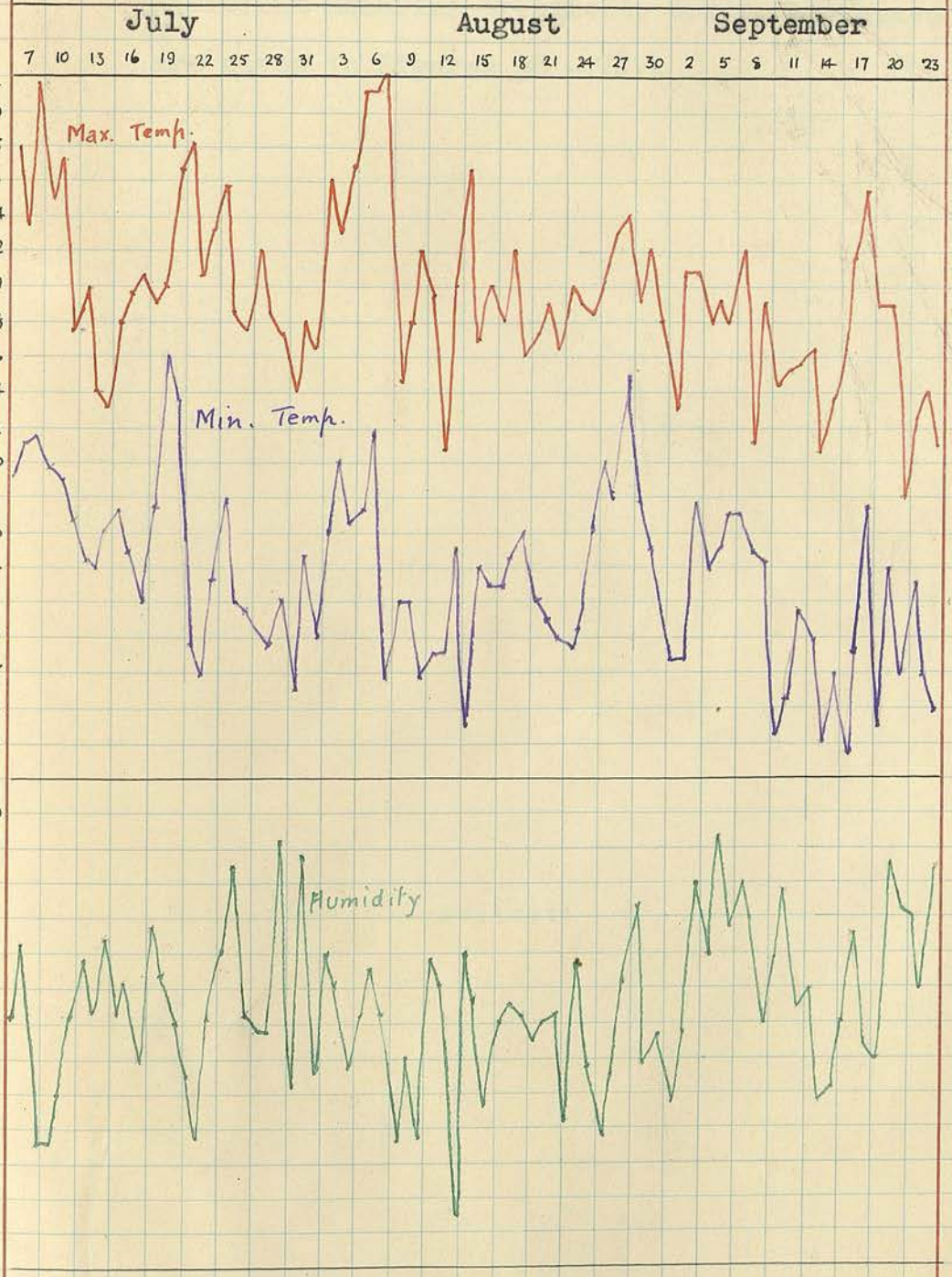


Fig 29

together with the dates of abscission of these on plants of a number of varieties which received different manurial treatments during two seasons are given in Figures 30 - 98.

::::::::

KEY TO ALL FIGURES.

↑↑ Number of flowers opened shown on the day indicated by cross-bars. Where more than 4 flowers opened on the same day, it is marked by one arrow and a note giving the number.

t Bud cluster first discernible.

5/3 Numbers in black:- daily counts of flower-buds dropped.

2/1 Numbers in red :- daily counts of flowers dropped.

⓪ Numbers in red circle:- daily counts of fruits dropped.

1 Line in black indicates that all flower-buds dropped.

2 Buds Left :- Flower-buds remaining after the plant had been killed by frost.

VARIETY - BRITISH QUEEN.

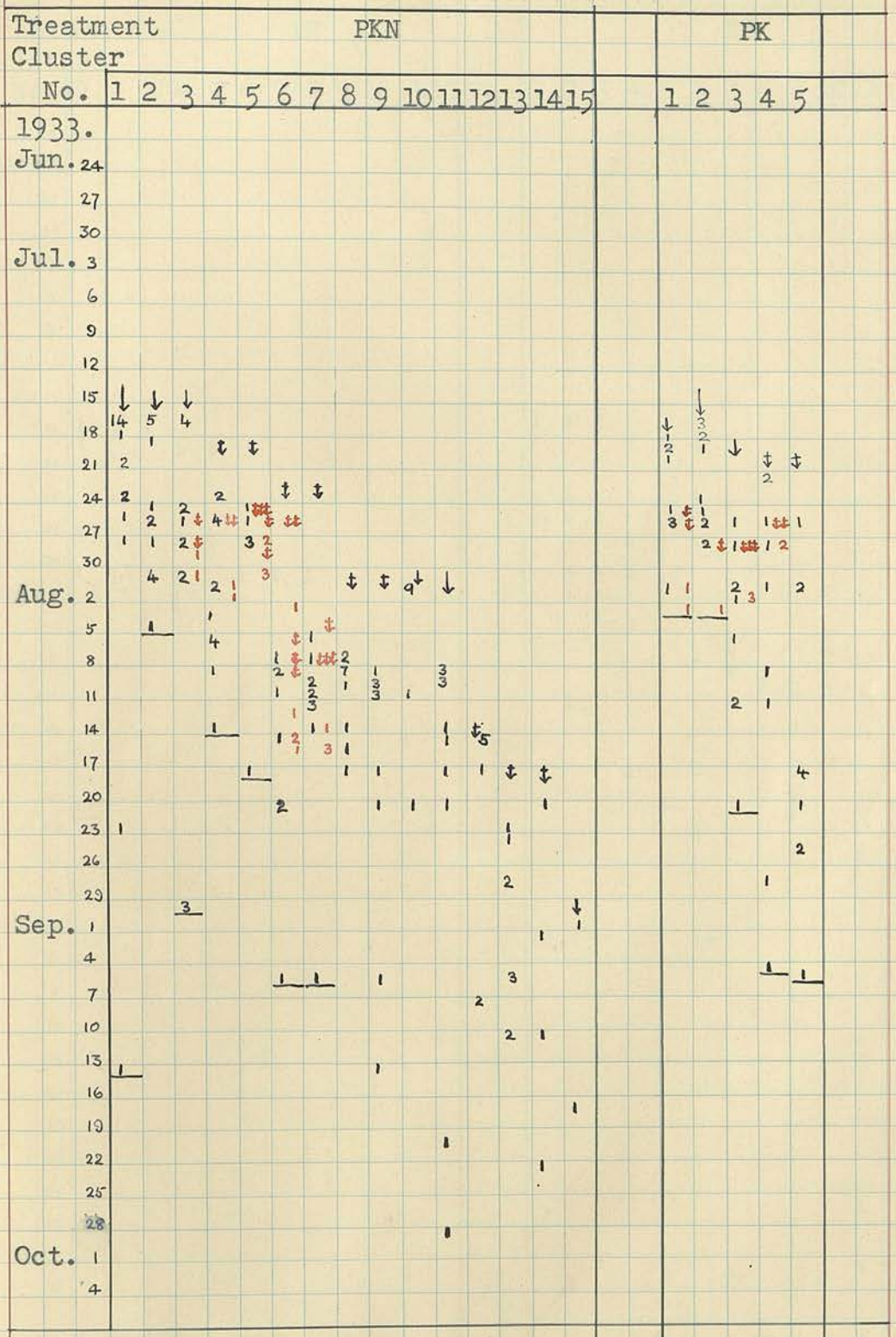


Fig. 30

Fig. 31

VARIETY - BRITISH QUEEN - Contd.

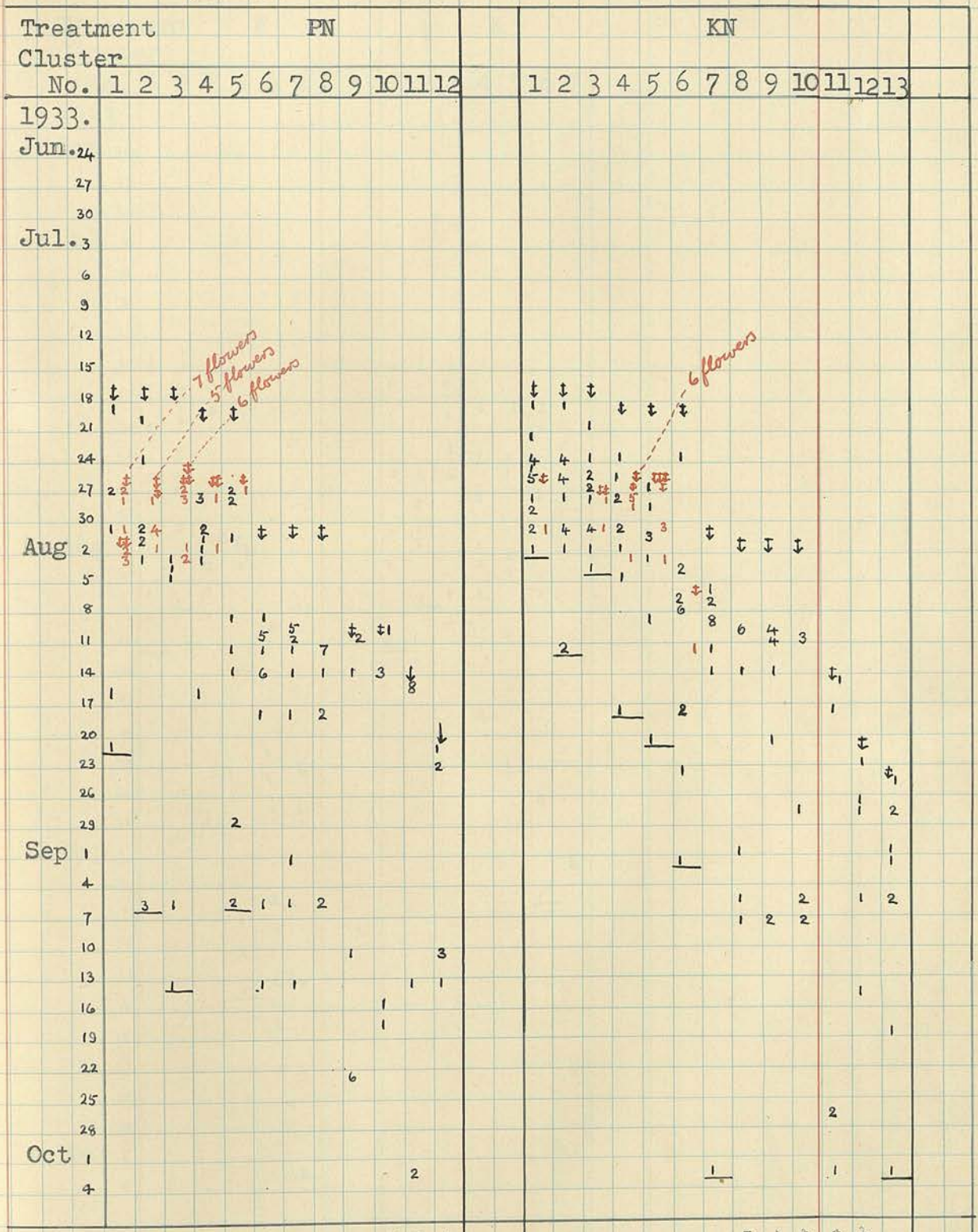


Fig. 32

Fig. 33

VARIETY - BRITISH QUEEN - Contd.

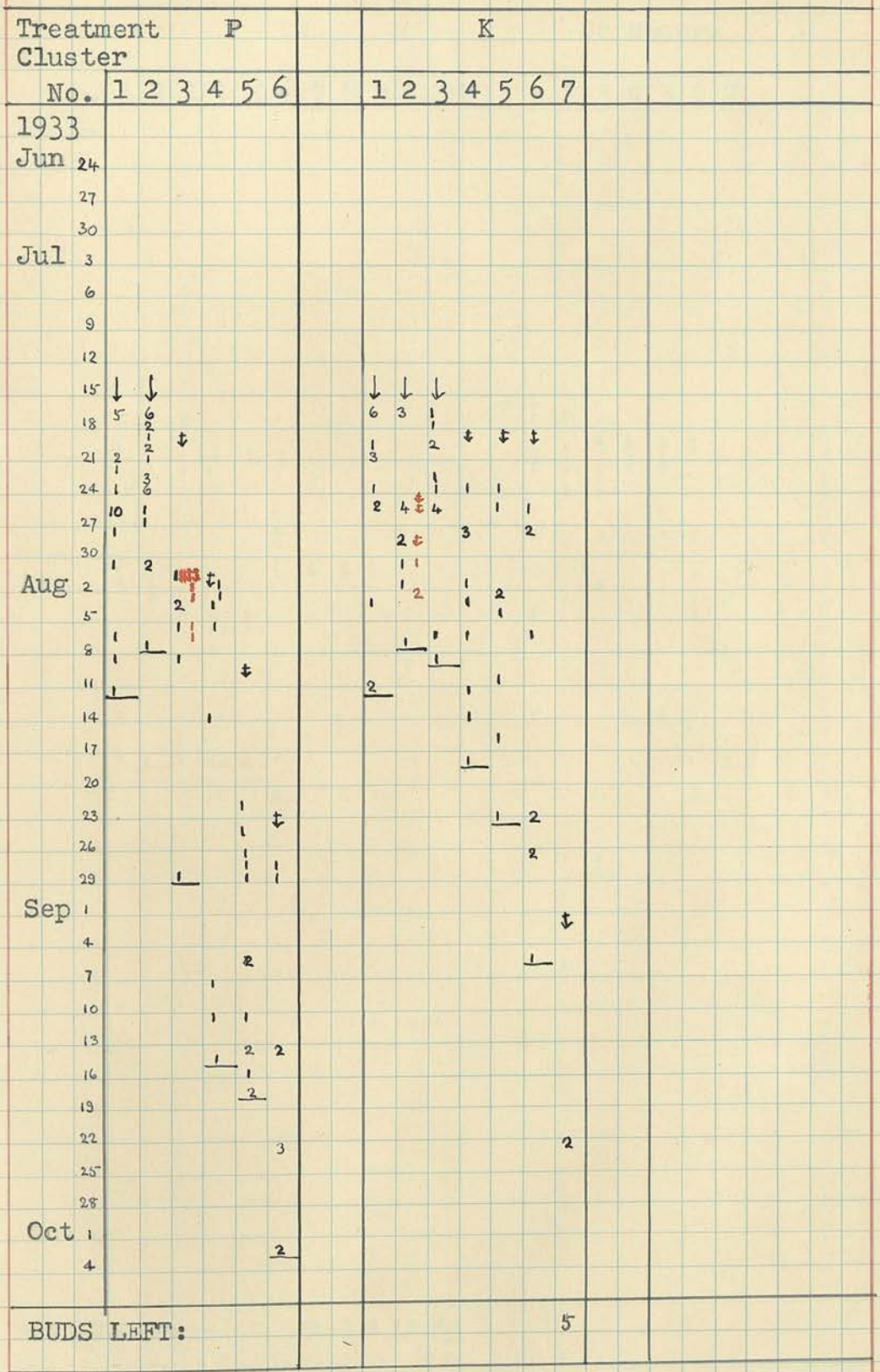


Fig. 34 Fig. 35

VARIETY - BRITISH QUEEN - Contd.

Treatment	N											No Manure						
Cluster No.	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7
1933.																		
Jun 24																		
27																		
30																		
Jul 3																		
6																		
9																		
12																		
15																		
18	↓	↓	↓									↓	↓	↓	↓	↓	↓	↓
21	2	1	2 1	↓	↓	↓						1 1 3 1	3 1 2	1 1 1	4 2	6 3	1	1
24	1	1	1	1		2							1	1				
27				↓	1	4 2						1 2		2		1	1	1
30	3 1 3 1	1 1 2 3	3 2 2	1		1	↓	↓	↓				3					
Aug 2		↓		↓	↓									2			1 2 3	
5	1	2		1 1 2	2 3 ↓	↓						1	1		2	1		3
8	1			2	1 1	1	4	4	2	↓					1			1
11				1	1		1	1	1									
14		1					1	1			↓ 1	1			1	2		
17		1	2	1	2	1	1		1	6							1	2
20						1		1	3					1			2	2
23											1							
26											1		1	1 1				
29																		
Sep 1									1									
4																		
7			1						1				1					
10							1		3		2							
13	1							1			2							
16																		
19										1								
22							2											
25																		
28																		
Oct 1																		
4									1									
BUDS LEFT:							4	3	2	3	4							

Fig. 36

Fig. 37

VARIETY - MAJESTIC.

Treatment	PKN											PK					
Cluster No.	1	2	3	4	5	6	7	8	9	10		1	2	3	4		
1933.																	
Jun 24																	
27																	
30																	
Jul 3																	
6																	
9																	
12																	
15																	
18	‡	‡										3					
21	1		‡											‡			
24	2	1	1									1		1			
27	1	1	‡														
30	4 2	3										3	1				
Aug 2	2	2	2	‡									1	‡	‡		
5	1	1	2 ‡ 2 ‡ 1											1			
8	1		1	1 3	‡	‡	‡					1		1			
11		1	1	1										1			
14	2		1	2			2						1 2				
17			6 1											1 2	1		
20			1										2	2	1		
23							1 3	‡									
26					1	1									1 2		
29					1				‡	‡		1	1				
Sep 1																	
4																	
7				4											2		
10															1		
13			1	2	2		1	1				2	1				
16							1	1				1			1		
19							2										
22						3		1									
25					1		3		1								
28					1												
Oct 1																	
4					2												
BUDS LEFT:				2	4		5	10	5								

Fig. 38

Fig. 39

VARIETY - MAJESTIC - Contd.

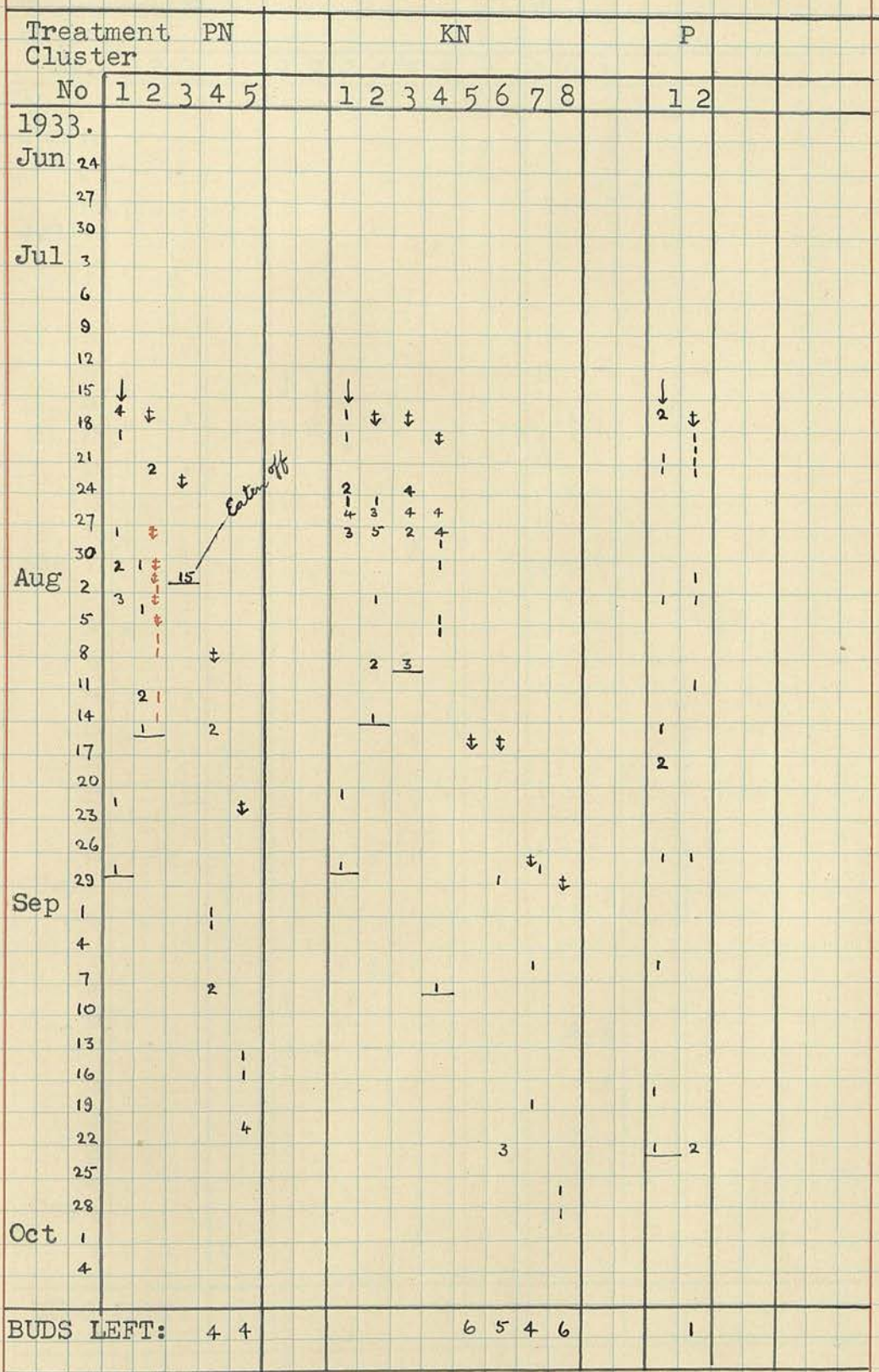


Fig. 40 Fig. 41 Fig. 42

VARIETY - MAJESTIC - Contd.

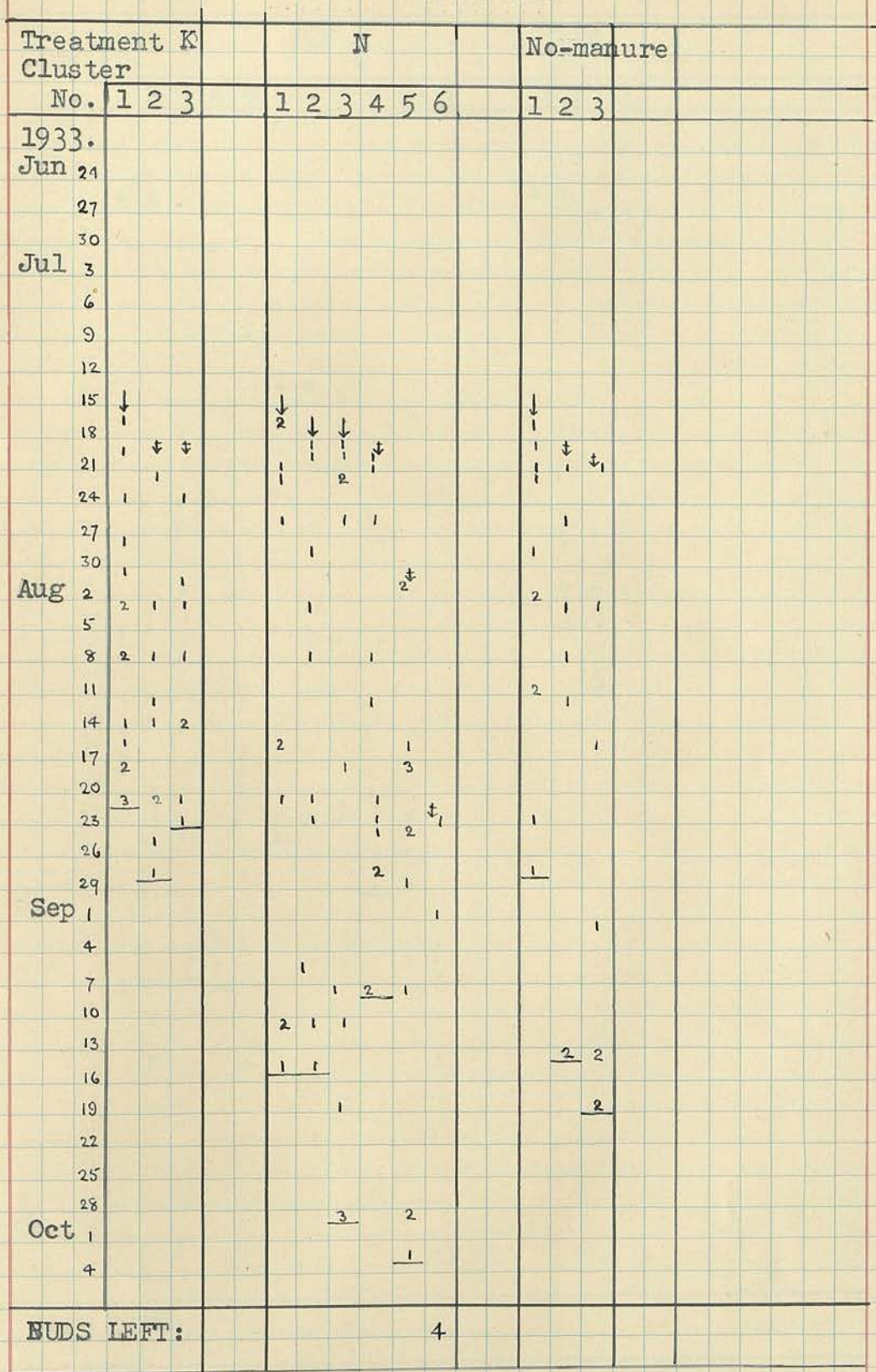
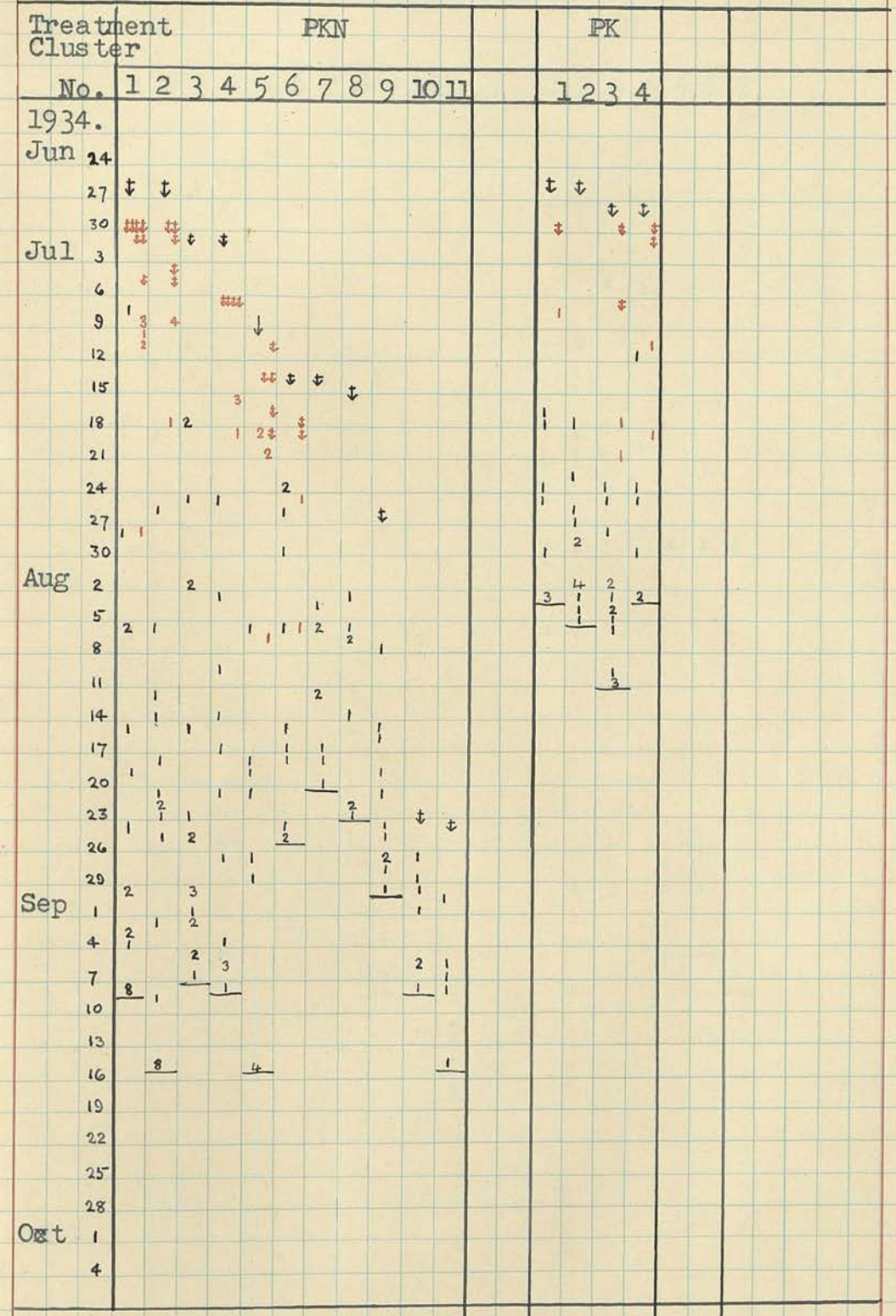


Fig. 43 Fig. 44 Fig. 45

VARIETY - CATRIONA.



BUDS LEFT:

Fig. 46

Fig. 47

VARIETY - CATRIONA - Contd.

Treatment Cluster No.	PN 1	2	3	4	5	6	7	8	9	10	11	KN 1	2	3	4	5	6	7
1934.																		
Jun 24																		
27	‡	‡	‡	5 flowers								‡	‡					
30			‡‡	‡														
Jul 3	‡		‡									‡‡						
6				‡	‡									‡	↓			
9												‡‡‡	‡‡‡			‡		
12	1	1	7	½	1		‡					1	3	1	‡	‡		
15		1		1				‡					2			1		
18	4	1	1		1			‡						2	1	1		
21	1				1								2		1			
24												1						
27			1	1					‡					1		2		
30			1	3									1			‡	‡	‡
Aug 2		1										1						
5			1									1						
8	3	1		2	2	2	2	1		‡		1	1		1			
11											‡			1				
14	1	1	1		1		1	1	1				1		1			
17	1			1	1			1	1					1		1		
20	1		1		1	1		1					1		1			
23				1		1										1		
26				1		1		3	1		1					1		1
29						1		1	1		1		2			1		1
Sep 1					2								2		3	2		
4						1			2	1	2		2		1			
7									2	1	1		8			2		2
10						1			2	1								2
13											1							1
16											2							
19											1							
22											3							
25																		
28																		
Oct 1																		
4																		
BUDS LEFT:																		

Fig. 48

Fig. 48

VARIETY - CATRIONA - Contd.

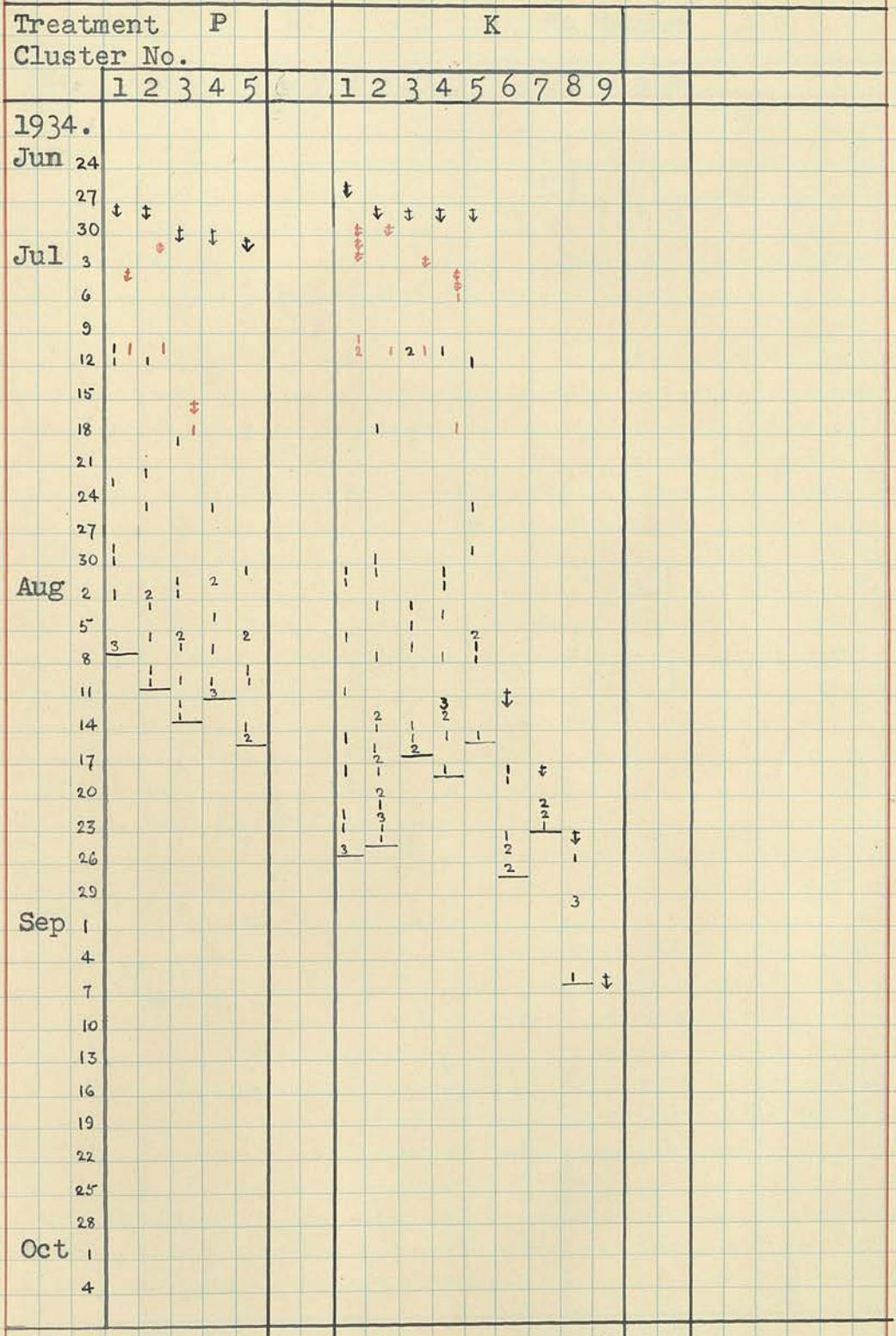
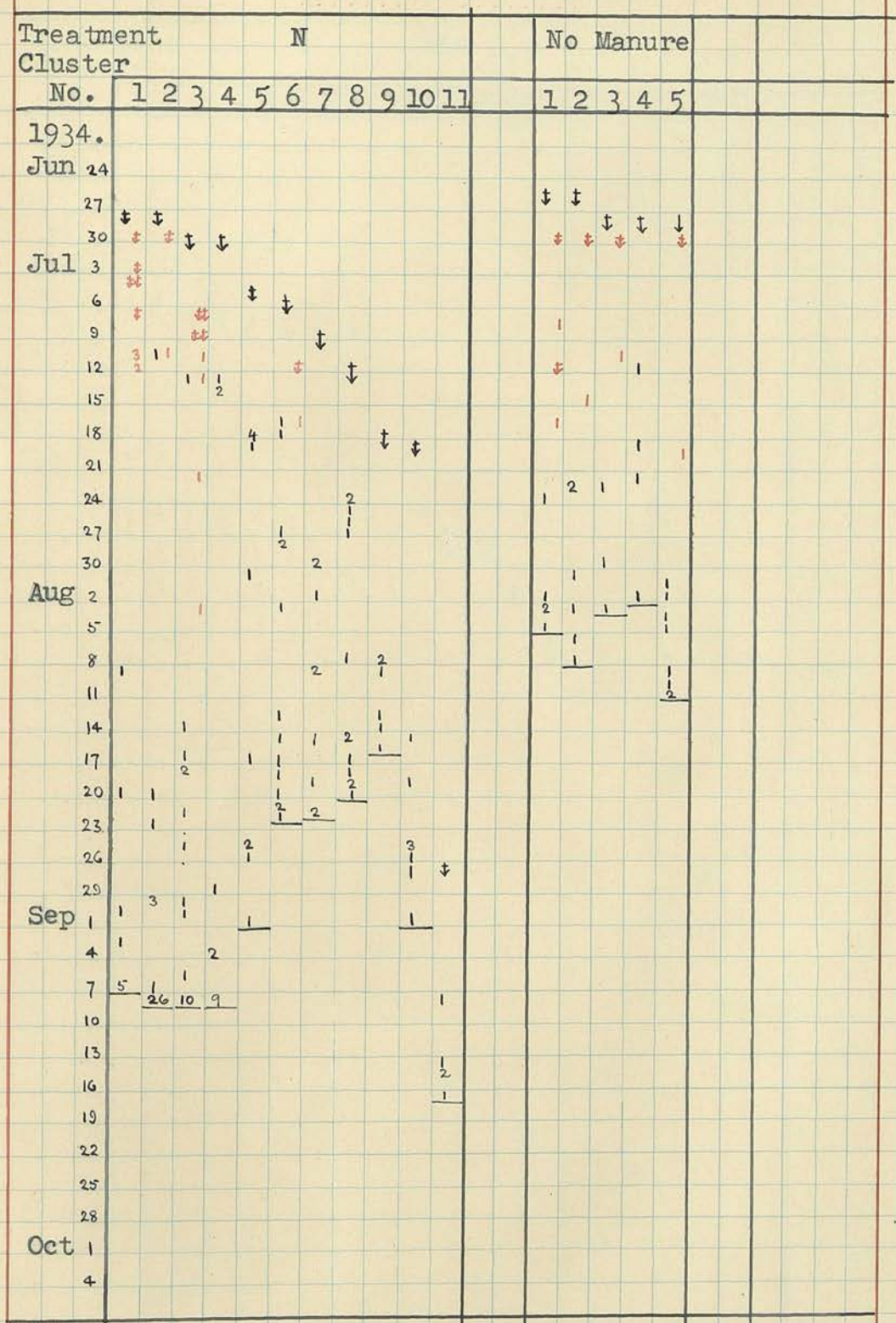


Fig. 49 Fig. 50

VARIETY - CATRIONA - Contd.



BUDS LEFT:

Fig. 51

Fig. 52

VARIETY - CHAMPION.

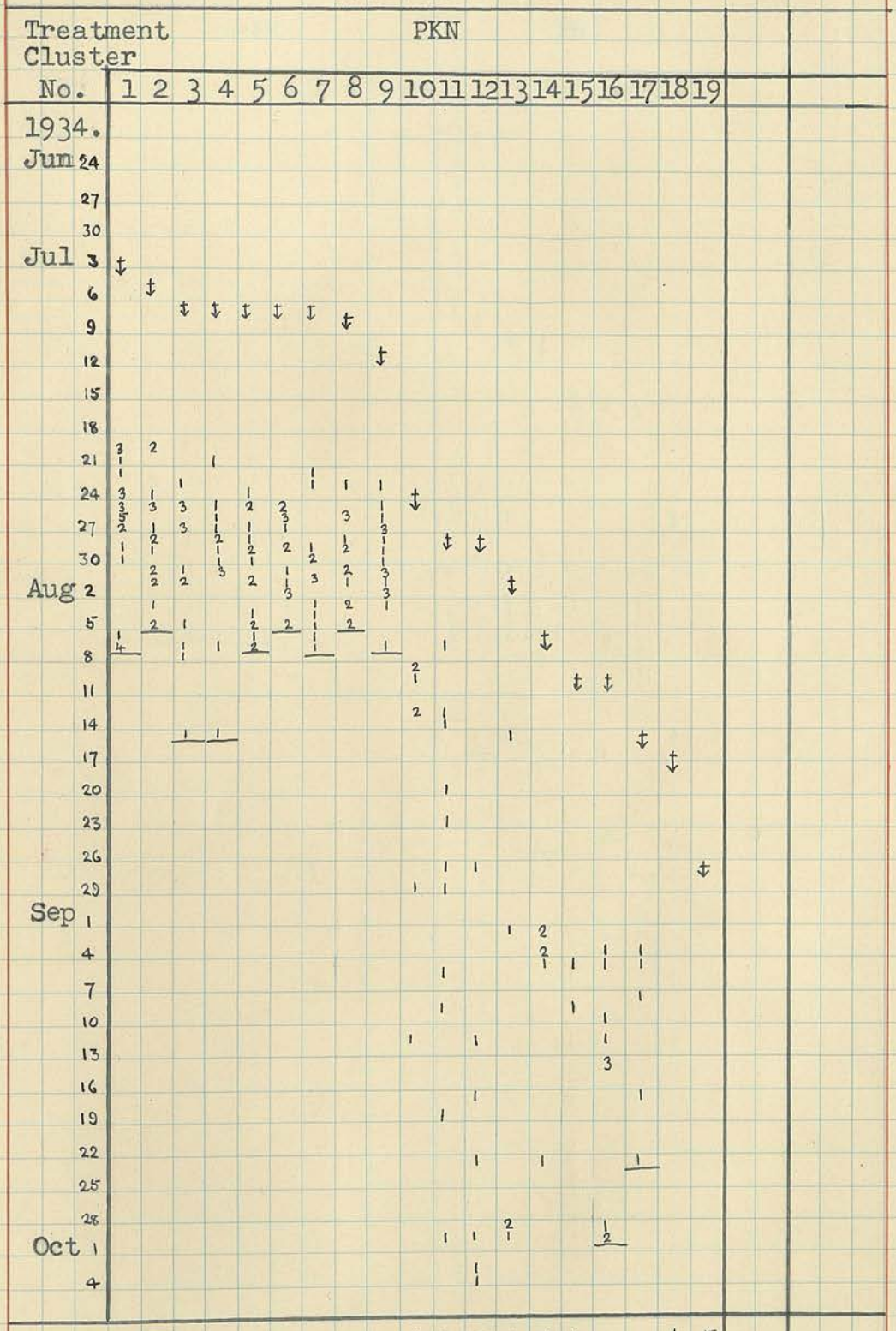


Fig. 53

VARIETY - CHAMPION - Contd.

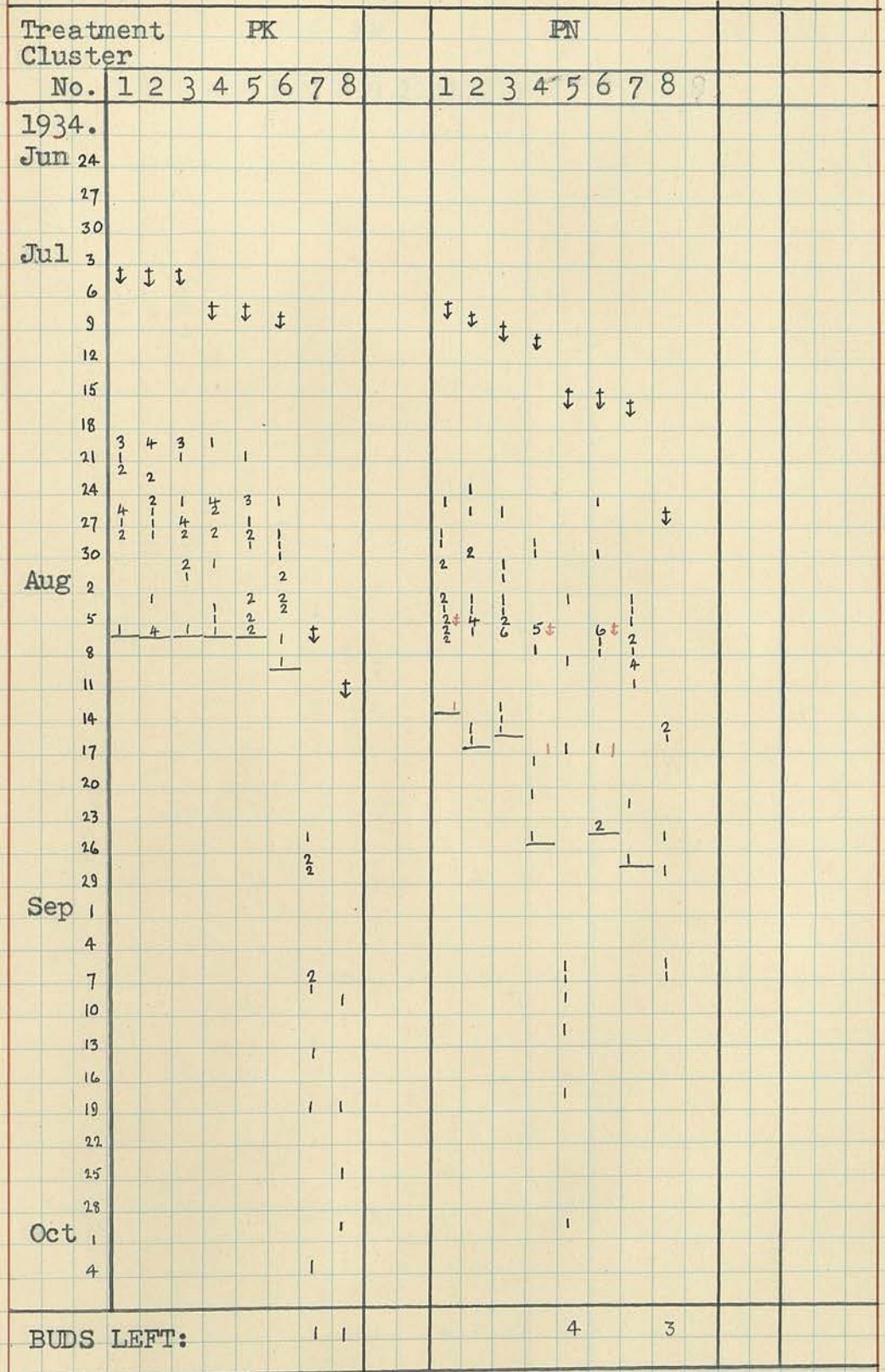


Fig. 54

Fig. 55

VARIETY - CHAMPION - Contd.

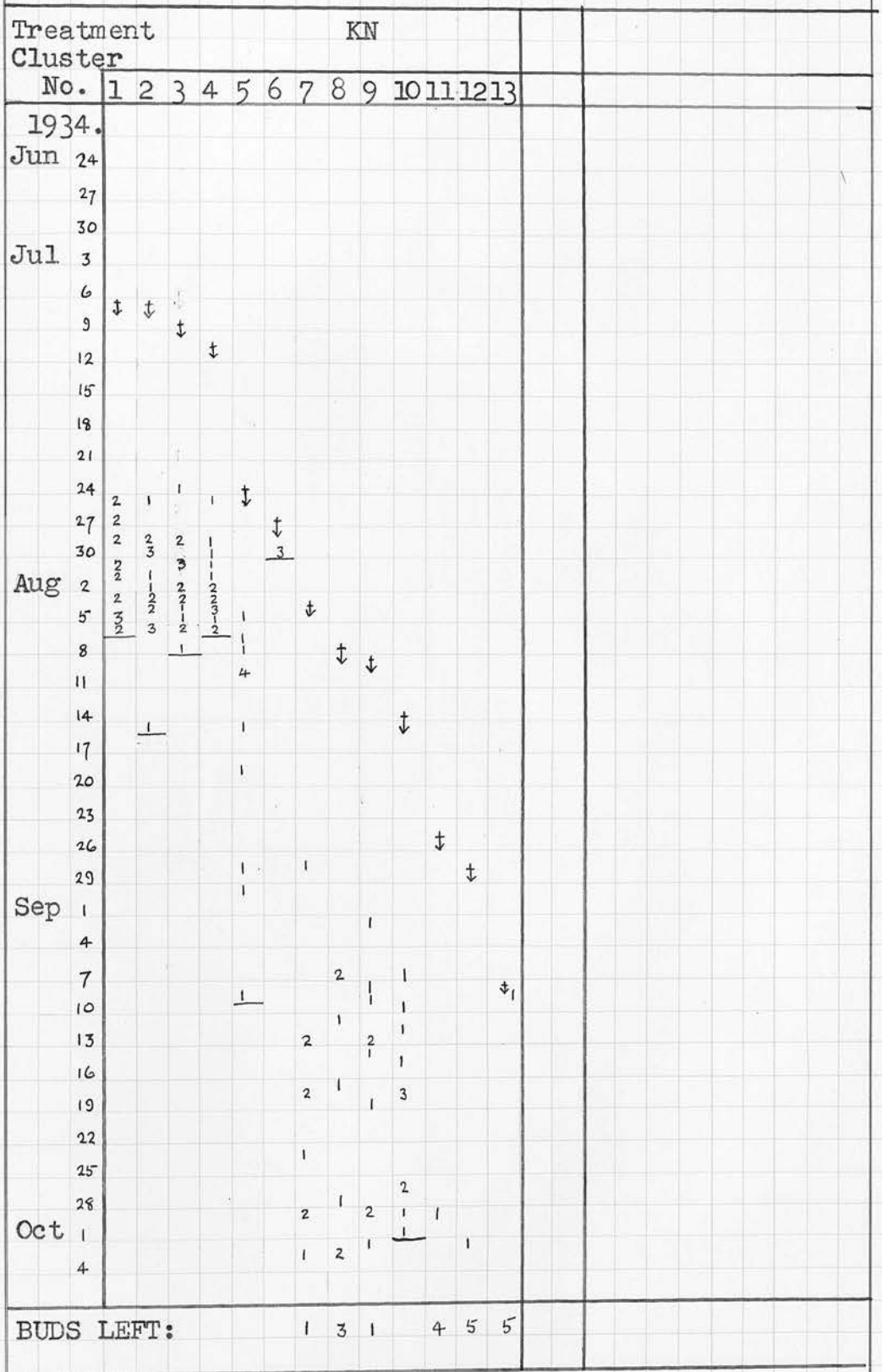


Fig. 56

VARIETY - CHAMPION - Contd.

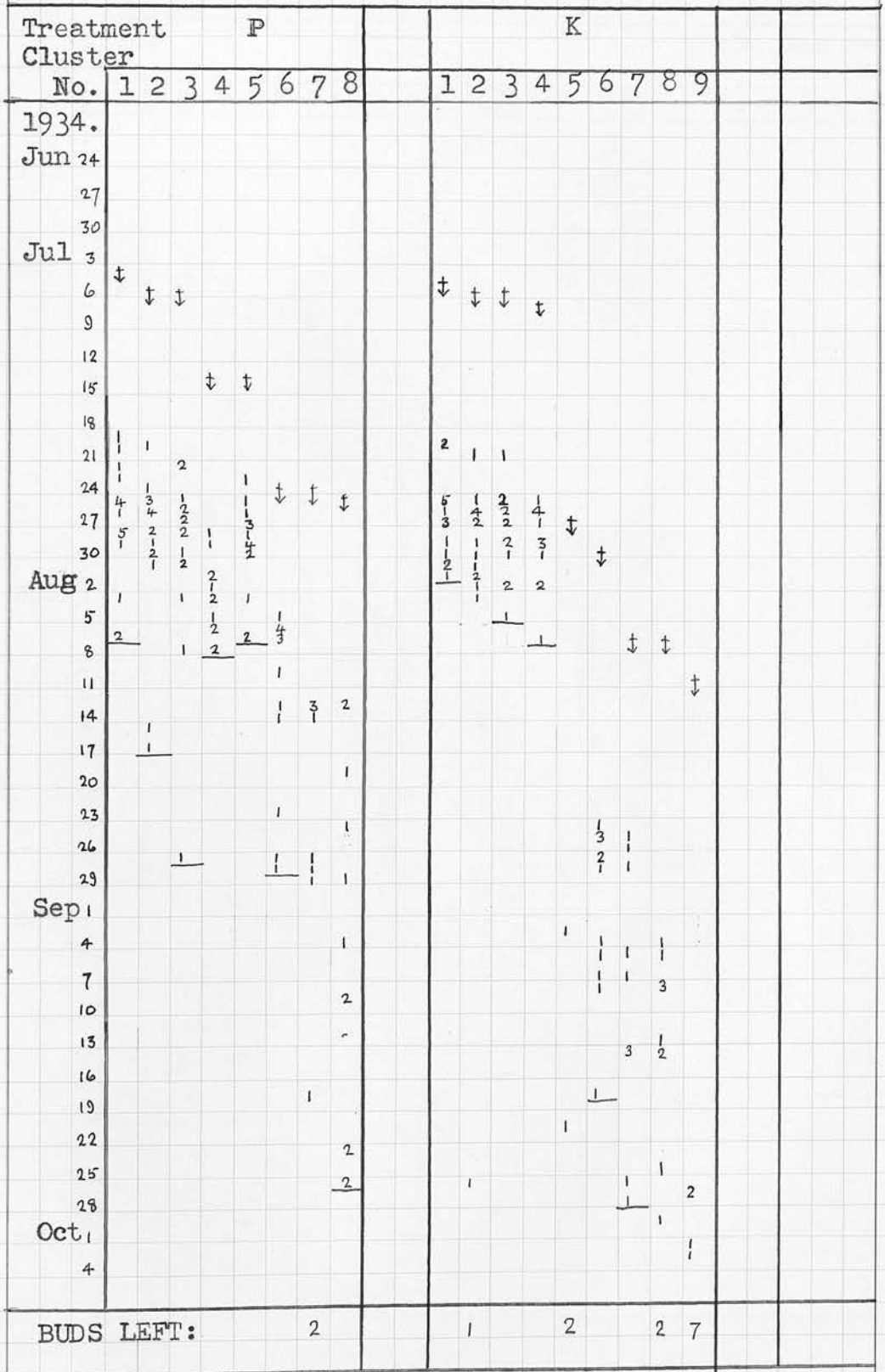


Fig. 57

Fig. 58

VARIETY - CHAMPION - Contd.

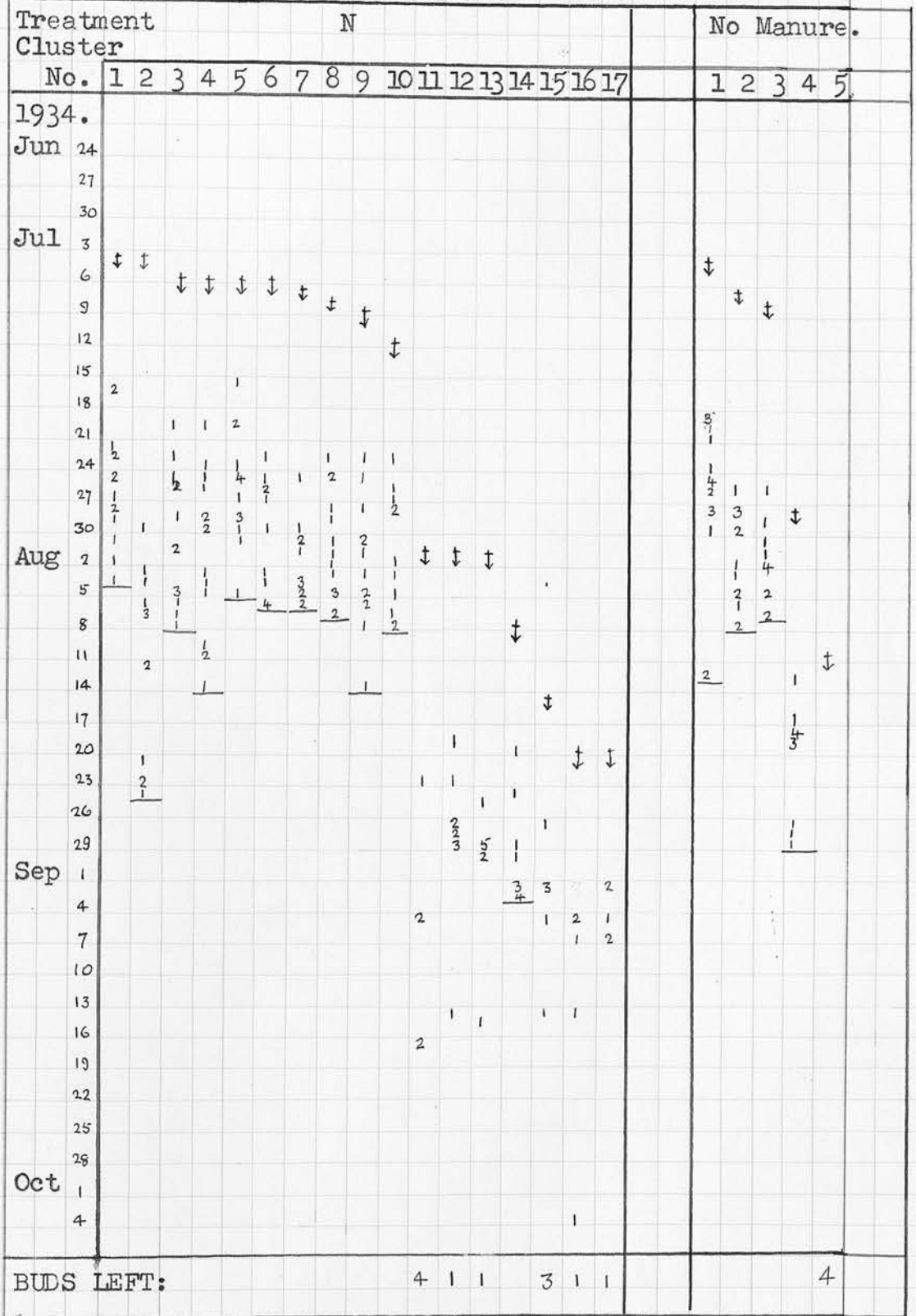


Fig. 59

Fig. 60

VARIETY - GREAT SCOT.

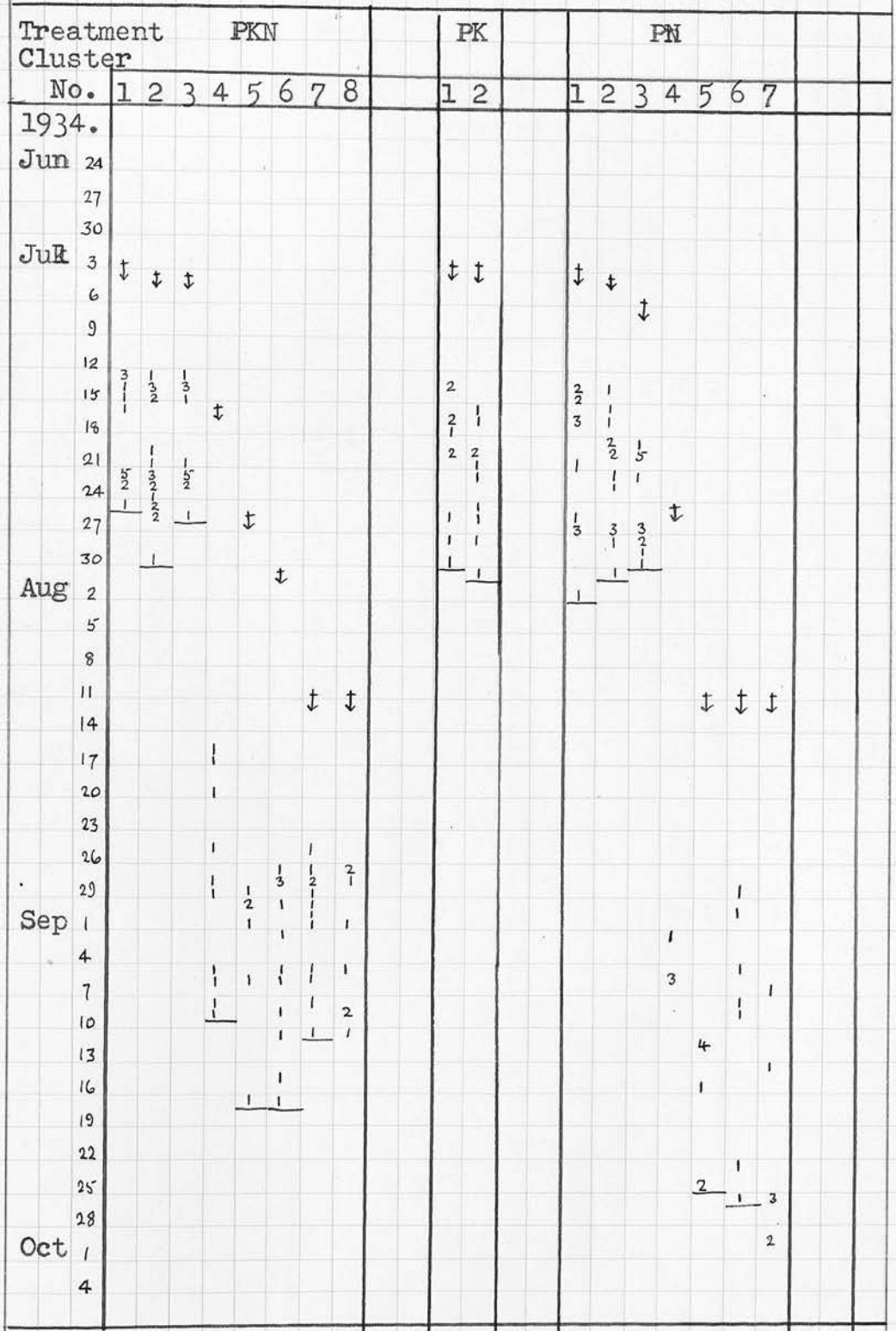


Fig. 61 Fig. 62 Fig. 63

VARIETY - GREAT SCOT - Contd.

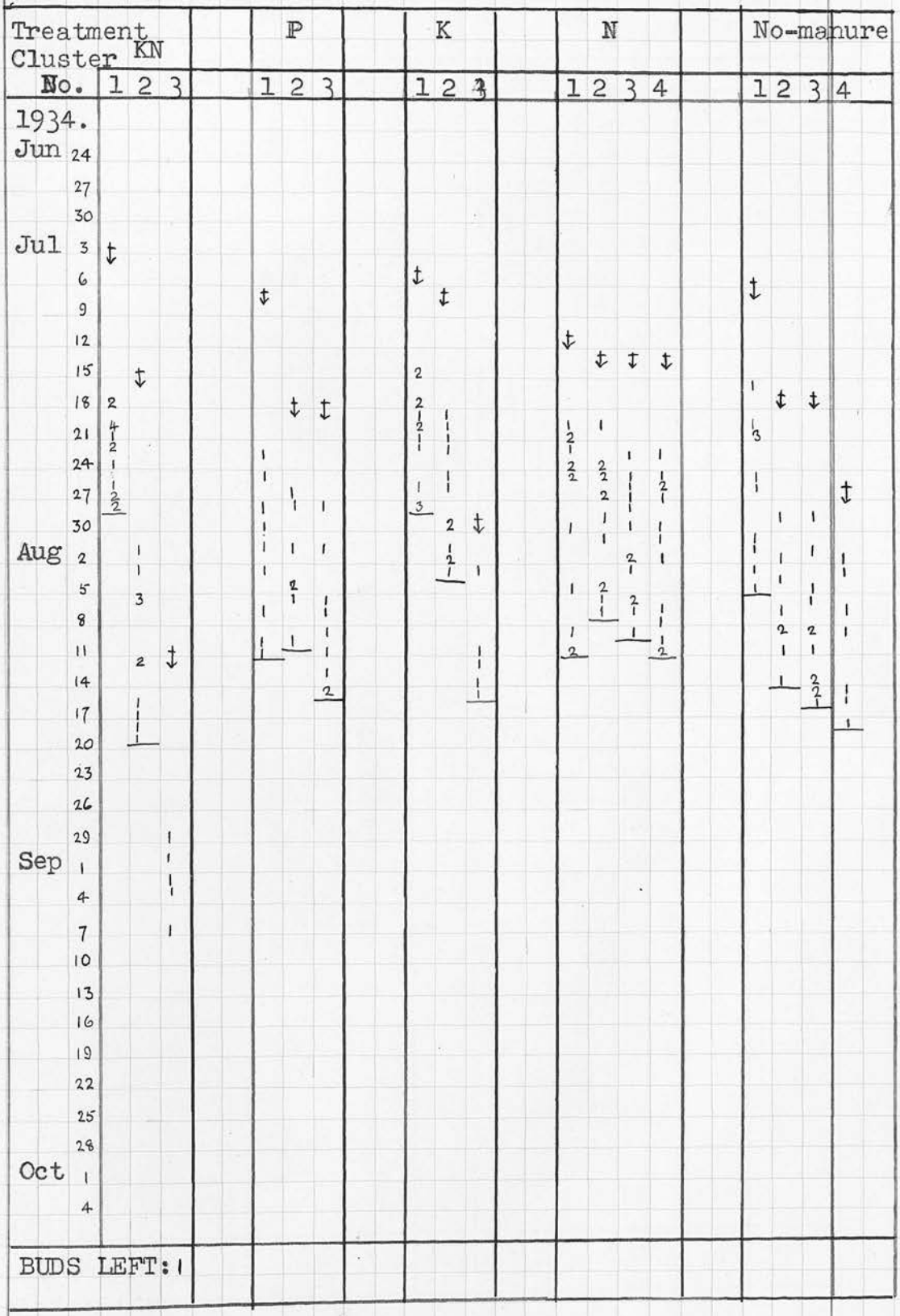


Fig. 64 Fig. 65 Fig. 66 Fig. 67 Fig. 68

VARIETY - GOLDEN WONDER.

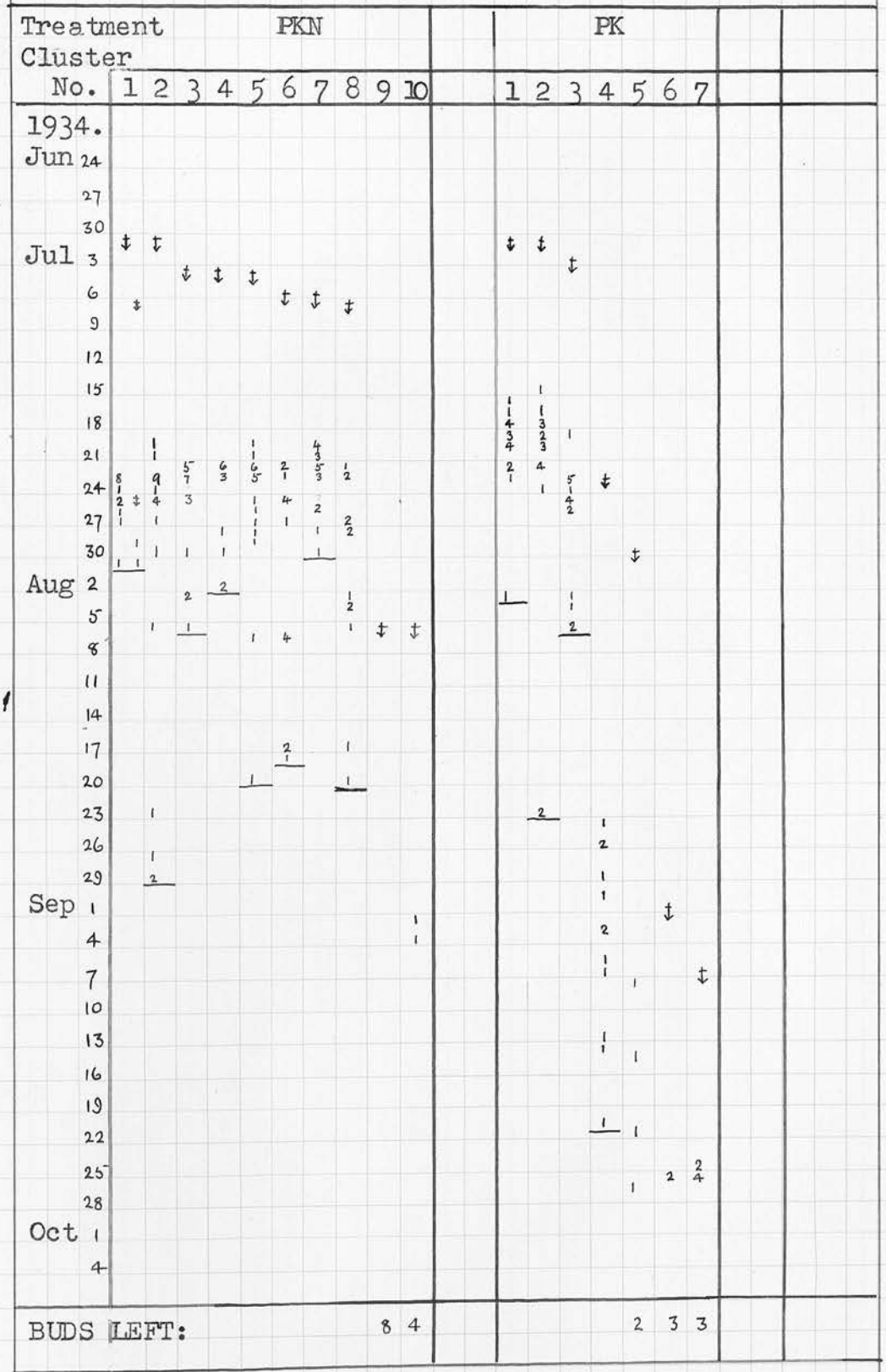


Fig. 69

Fig. 70

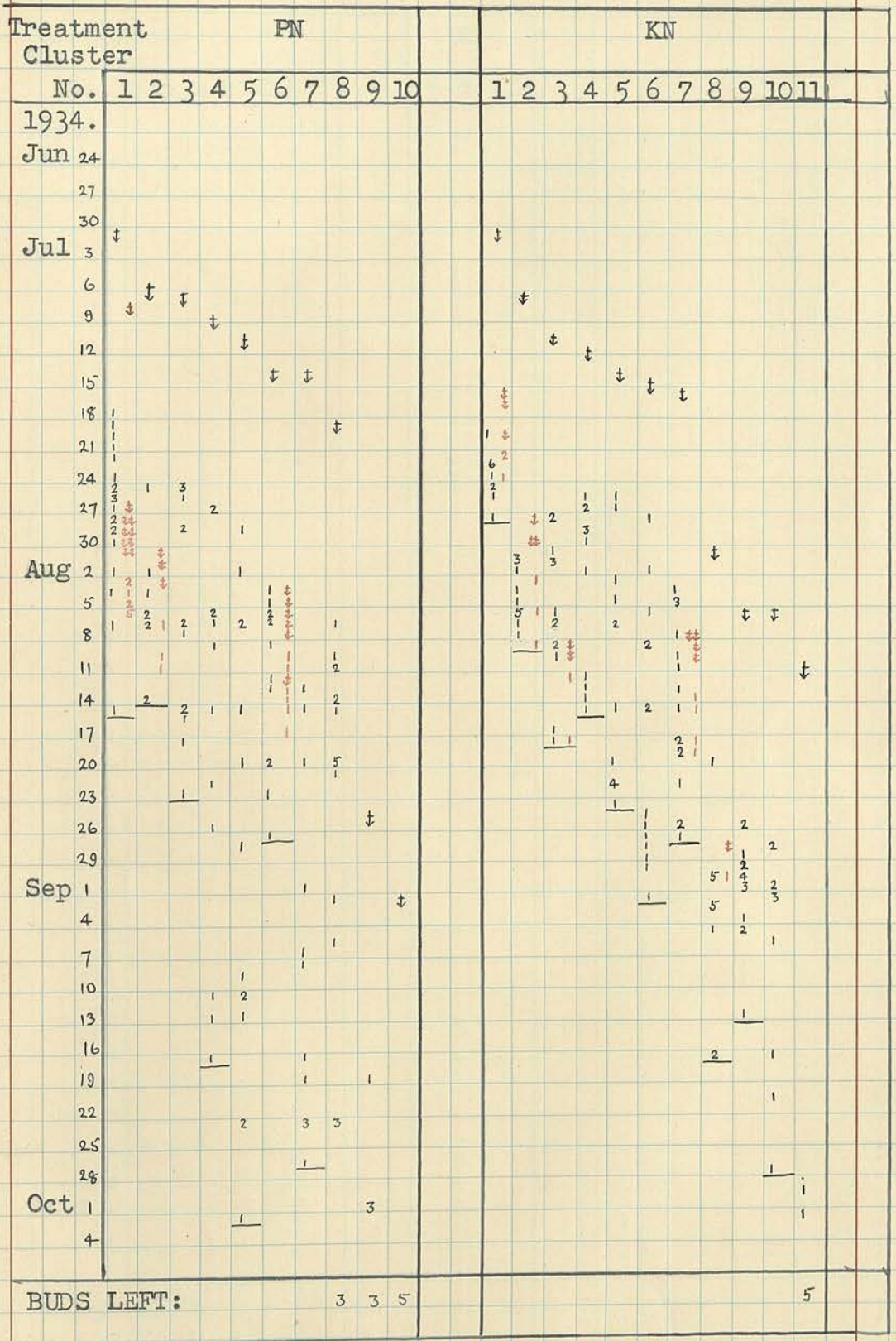

Treatment
Cluster
PN
KN
No.
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10 11
1934.
Jun 24
27
30
Jul 3
6
9
12
15
18
21
24
27
30
Aug 2
5
8
11
14
17
20
23
26
29
Sep 1
4
7
10
13
16
19
22
25
28
Oct 1
4
BUDS LEFT: 3 3 5
5

Fig. 71

Fig. 72

VARIETY - GOLDEN WONDER - Contd.

Treatment		P						K			
Cluster No.		1	2	3	4	5	6	1	2	3	4
1934.											
Jun	24										
	27										
	30	‡	‡								
Jul	3			‡				‡	‡	‡	‡
	6				‡						
	9										
	12										
	15										
	18	2 1 3	1 2		1 2			1 1 3 2	1		
	21	2 3	1 3 4	1 4 2		‡	‡	1	1 4 4	1 4 1	1 2 2
	24	3 1	3					1		2	1 1
	27		1 1	1 1 2					1		
	30	1						1			
Aug	2		2	1 1	2			4 1 1	1	1 2 1	1
	5			2							1
	8				1						1
	11										
	14				1						
	17										1
	20					1					2 1
	23				1						
	26				1 1	1 1					
	29				1 1	3 2					
Sep	1				1	1 1 1 2 1					
	4										
	7					1	1				
	10										
	13										
	16										
	19										
	22										
	25						1				
	28										
Oct	1										
	4										
BUDS LEFT:						1	4				

Fig. 73 Fig. 74

VARIETY - GOLDEN WONDER - Contd.

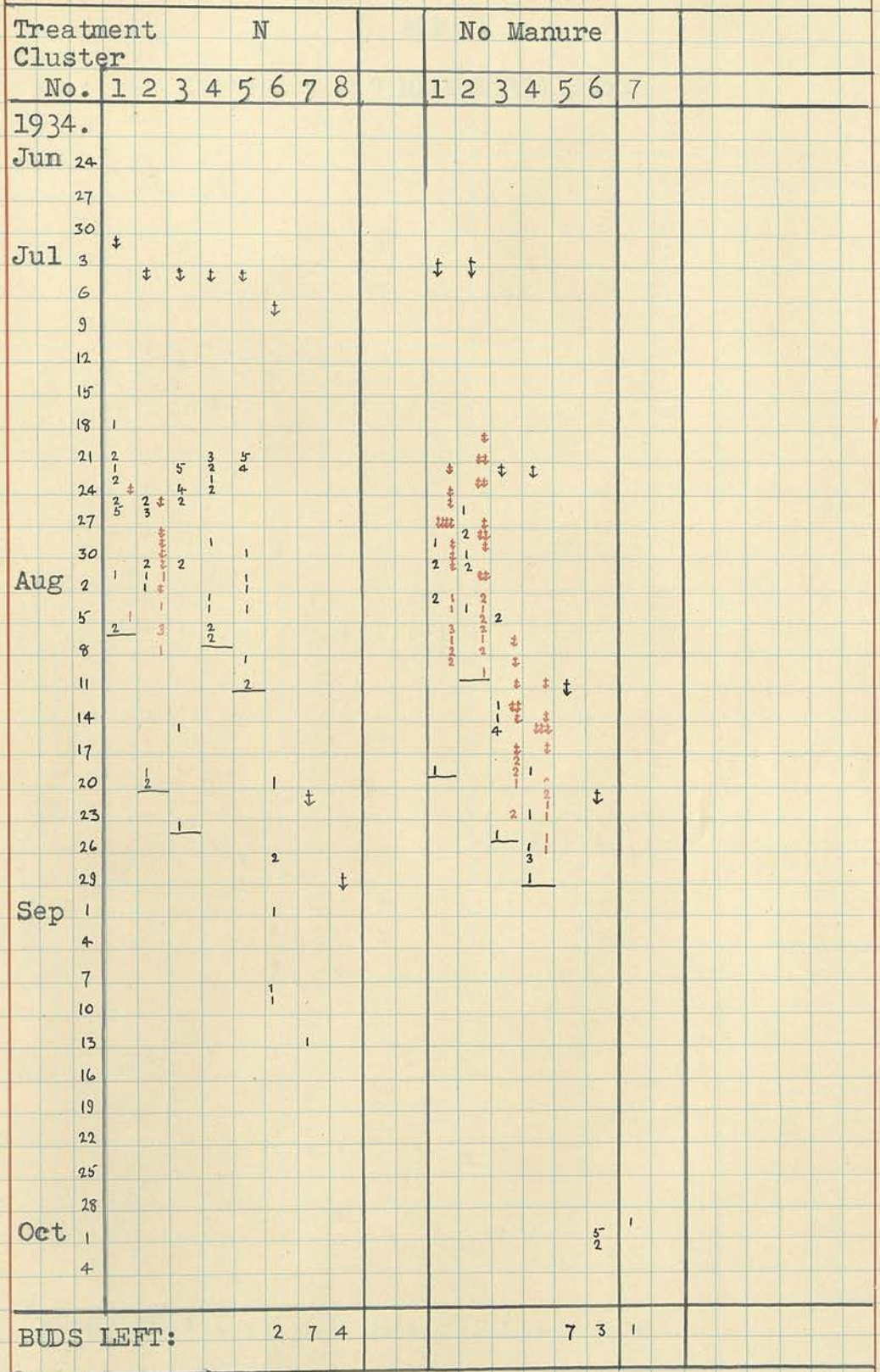


Fig. 75 Fig. 76

VARIETY - KING EDWARD.

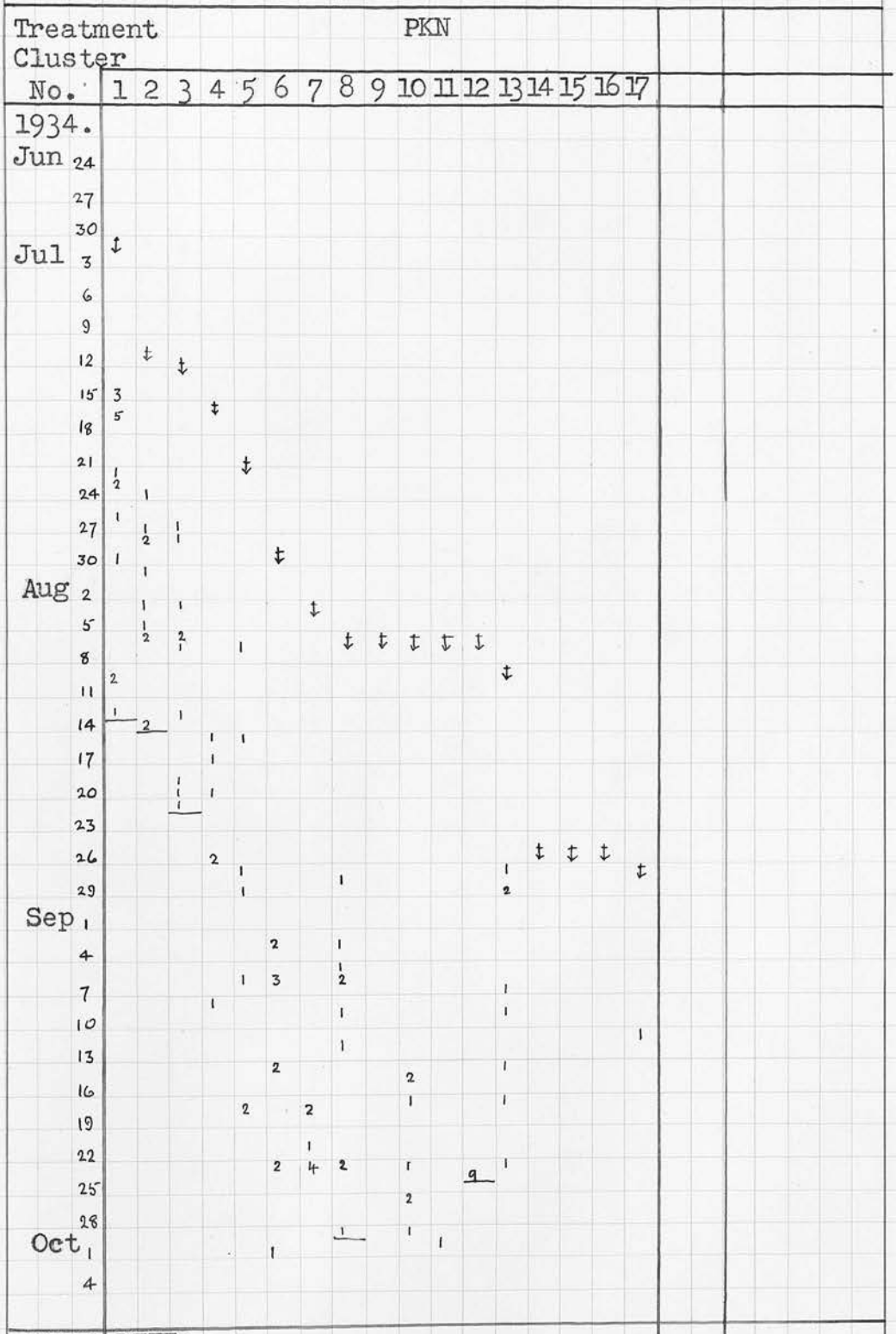


Fig. 77

Treatment Cluster No.	PK 1	PK 2	PK 3	PK 4	PK 5	PK 6	PK 7	PK 8	PK 9	PN 1	PN 2	PN 3	PN 4	PN 5	PN 6	PN 7	PN 8
1934 Jun 24																	
27																	
30	‡	‡	‡							‡	‡	‡					
Jul 3																	
6																	
9																	
12		1															
15	2 3	4 1 1	2 2	‡						1	1 1 1	1 1					
18	2 1 1	2 1 1	5							1 3	5 2	6					
21	3		2							5 1	2	2 1 1					
24	1				‡					1	2		‡				
27		1				‡								‡	‡		
30											1	1					
Aug 2	1	1	1	1			‡				1	1					
5		1						‡		1	1					‡	
8																	‡
11									‡								
14			1	2								1					
17																	
20				1							1						
23				1 1	1			2 1 1									
26																	
29				1	3		1										1 1
Sep 1																	
4					3	1 1 1	1 1 1							1			
7				1		1								1 1 1	1		1 1
10									1				1				
13						1	2										2
16									1				1		1		
19																	
22													1			1	
25														1			
28																	
Oct 1																	
4																	
BUDS LEFT:						3	2		8				6	4	7	10	1

Fig. 78

Fig. 79

VARIETY - KING EDWARD - Contd.

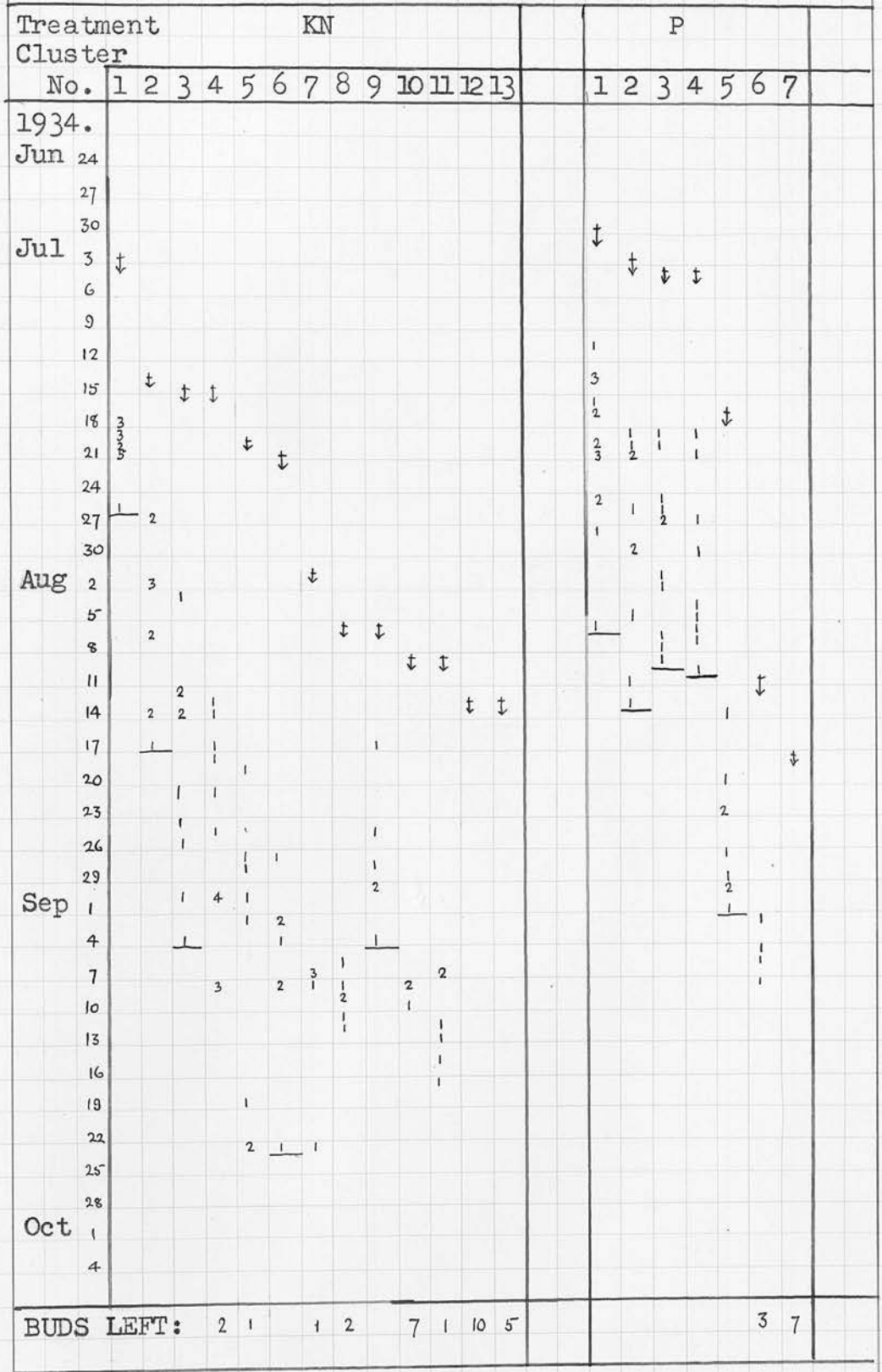


Fig. 80

Fig. 81

VARIETY - KING EDWARD - Contd.

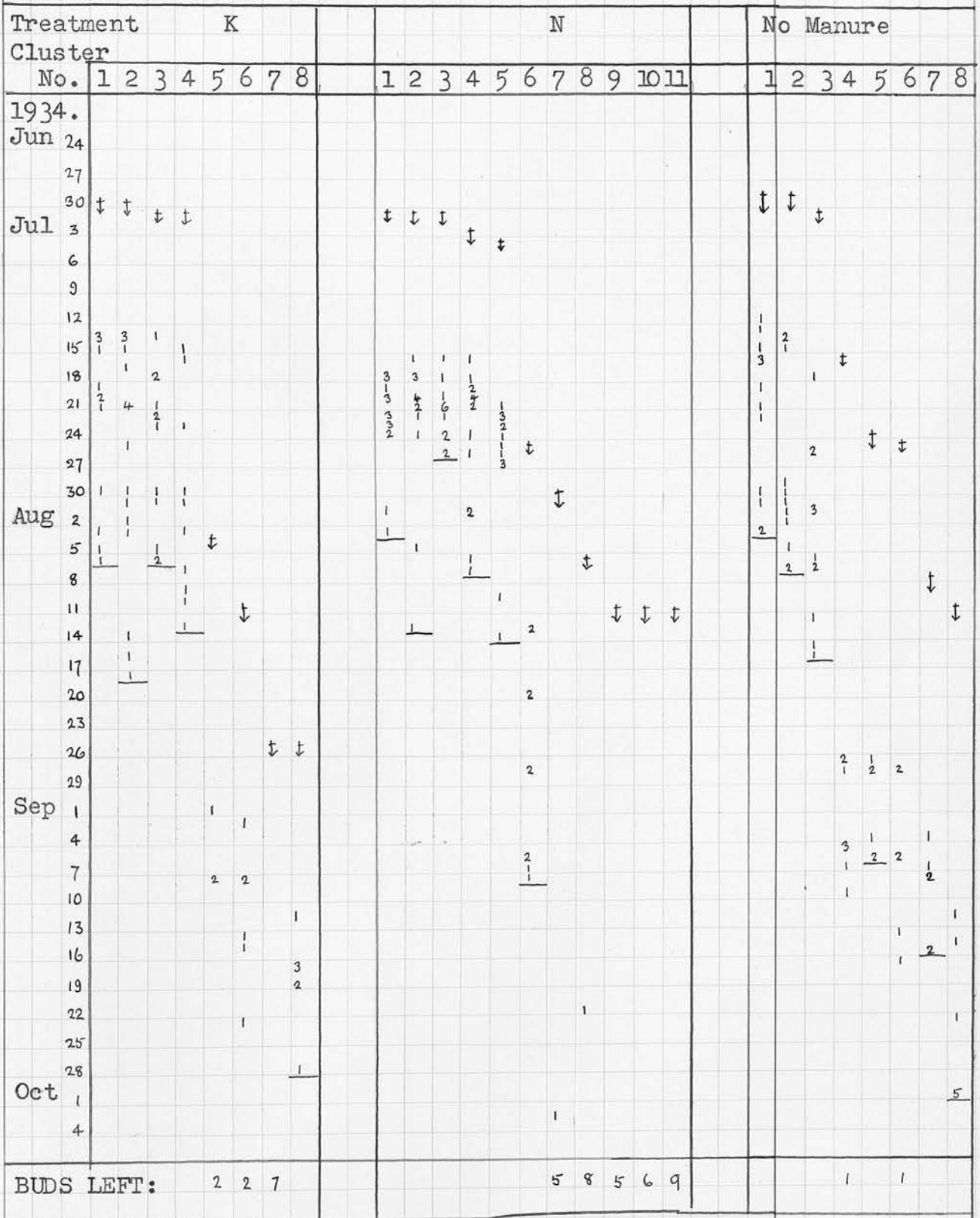


Fig. 82 Fig. 83 Fig. 84

VARIETY - MAJESTIC.

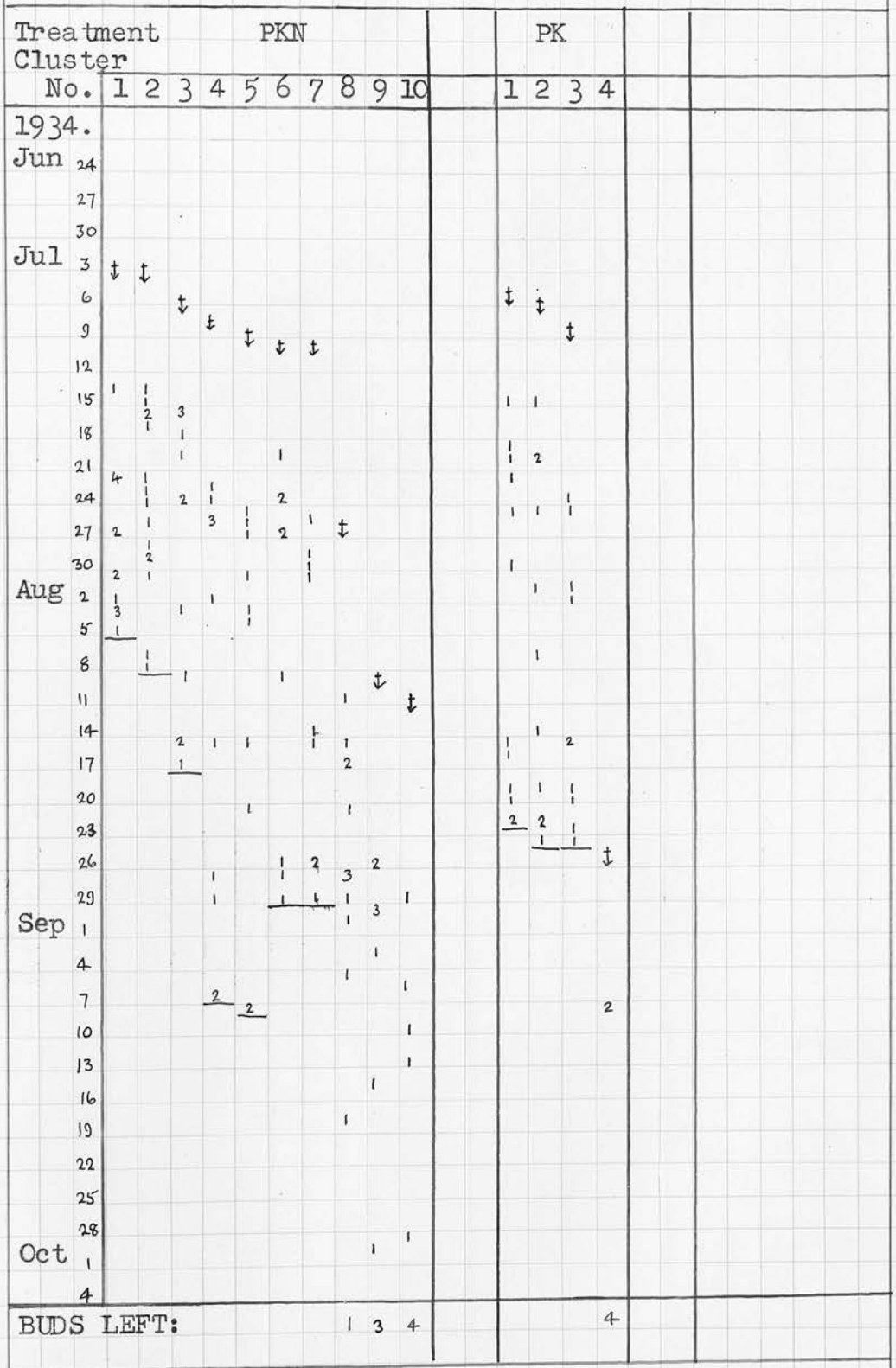


Fig. 85

Fig. 86

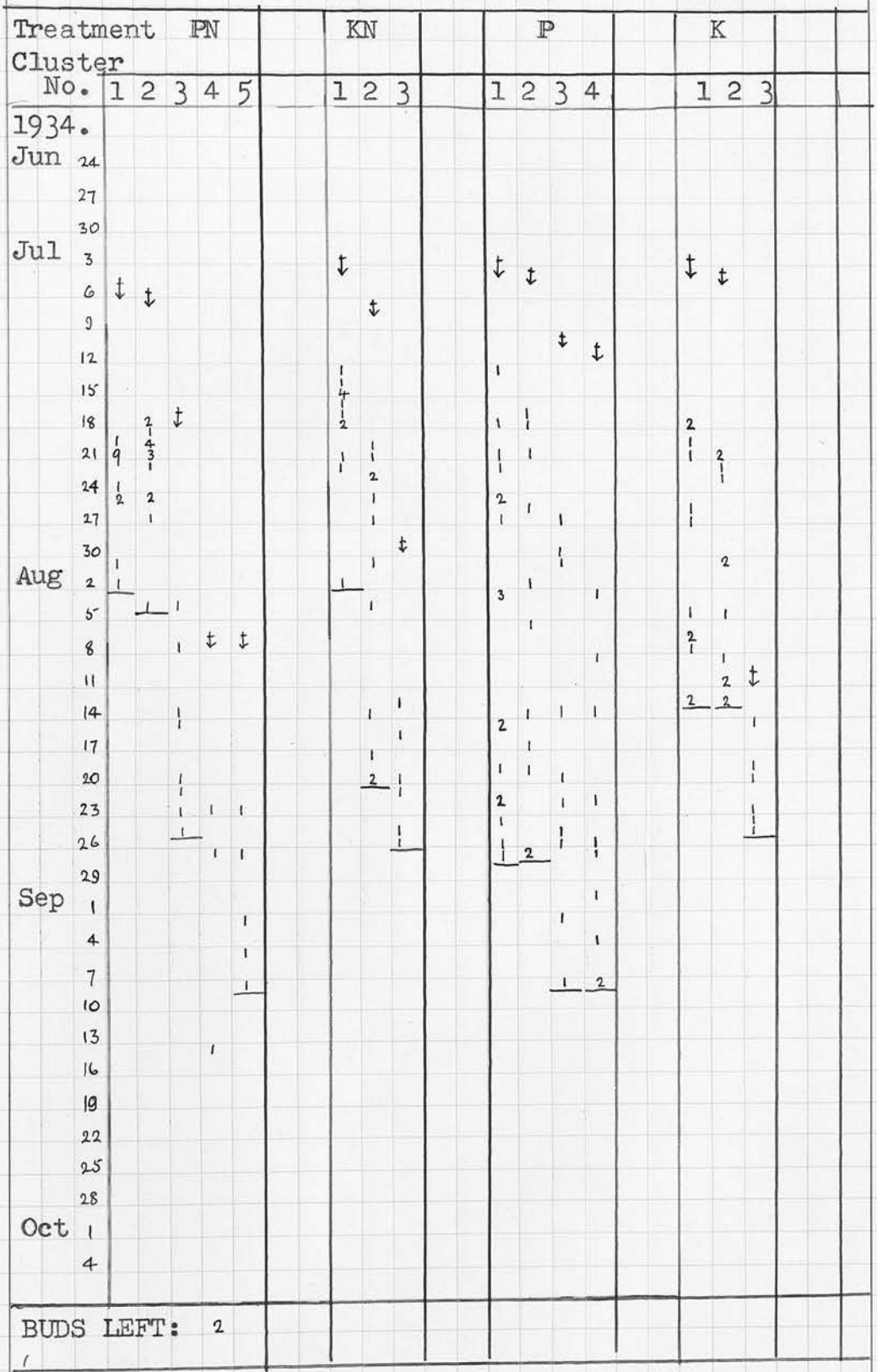


Fig. 87 Fig. 88 Fig. 89 Fig. 90

VARIETY - MAJESTIC - Contd.

Treatment	N					No-manure				
Cluster No.	1	2	3	4		1	2	3	4	
1934.										
Jun 24										
27										
30										
Jul 3	‡	‡								
6			‡							
9						‡				
12							‡	‡	‡	
15	1	4	2							
18	1 5	1	2					1		
21	1 1	2	1			1 1	1			
24		1				1	2			
27	1	1	2				2		1	
30				‡		1		1	1	
Aug 2	1		1				2	1	1	
5						2	2		1	
8			1							
11	1	2							1	
14			1						2	
17									2	
20				1						
23										
26				1						
29				1						
Sep 1				1						
4				1						
7				2 1						
10										
13										
16										
19										
22										
25										
28										
Oct 1										
4										
BUDS LEFT:				1						

Fig. 91

Fig. 92

SUMMARY

TOTAL DAILY COUNTS OF FLOWER-BUDS DROPPED ON 48 PLANTS (6 varieties each with 8 Manurial Treatments).

Key to Treatments :- Superphosphate = P
Sulphate of Potash = K
Sulphate of Ammonia = N

a = PKN	e = P
b = PK	f = K
c = PN	g = N
d = KN	h = No manure.

Fig. 93

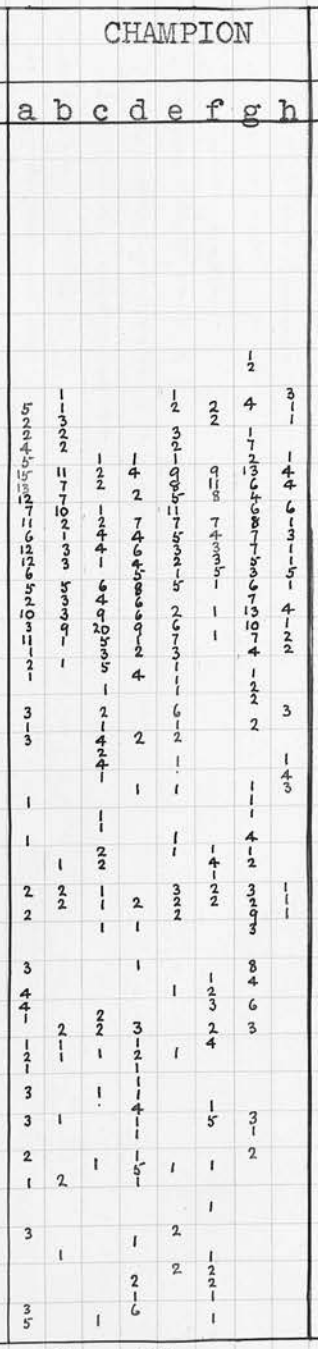


Fig. 94

SUMMARY - Contd.

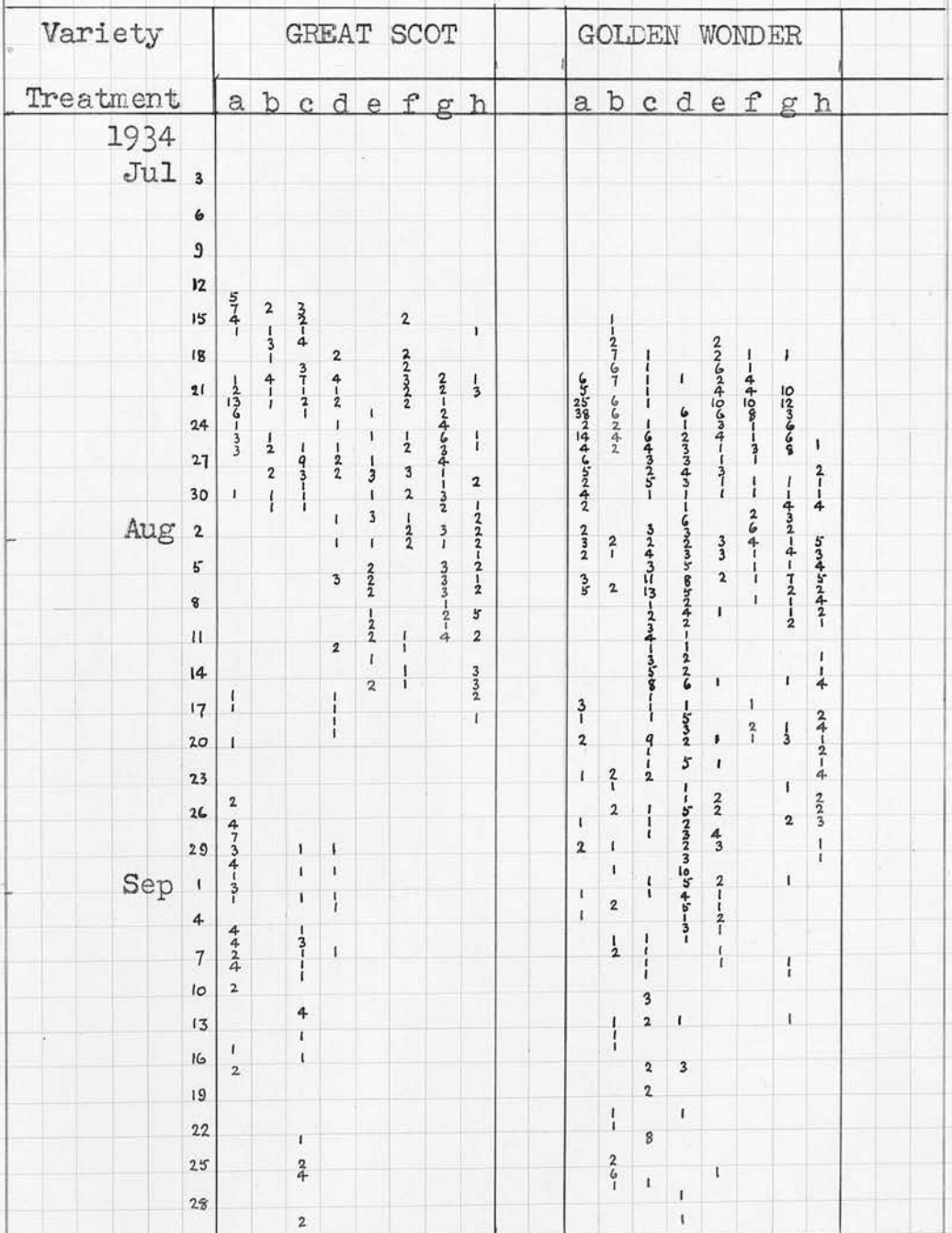


Fig.95 Fig. 96

SUMMARY - Contd.

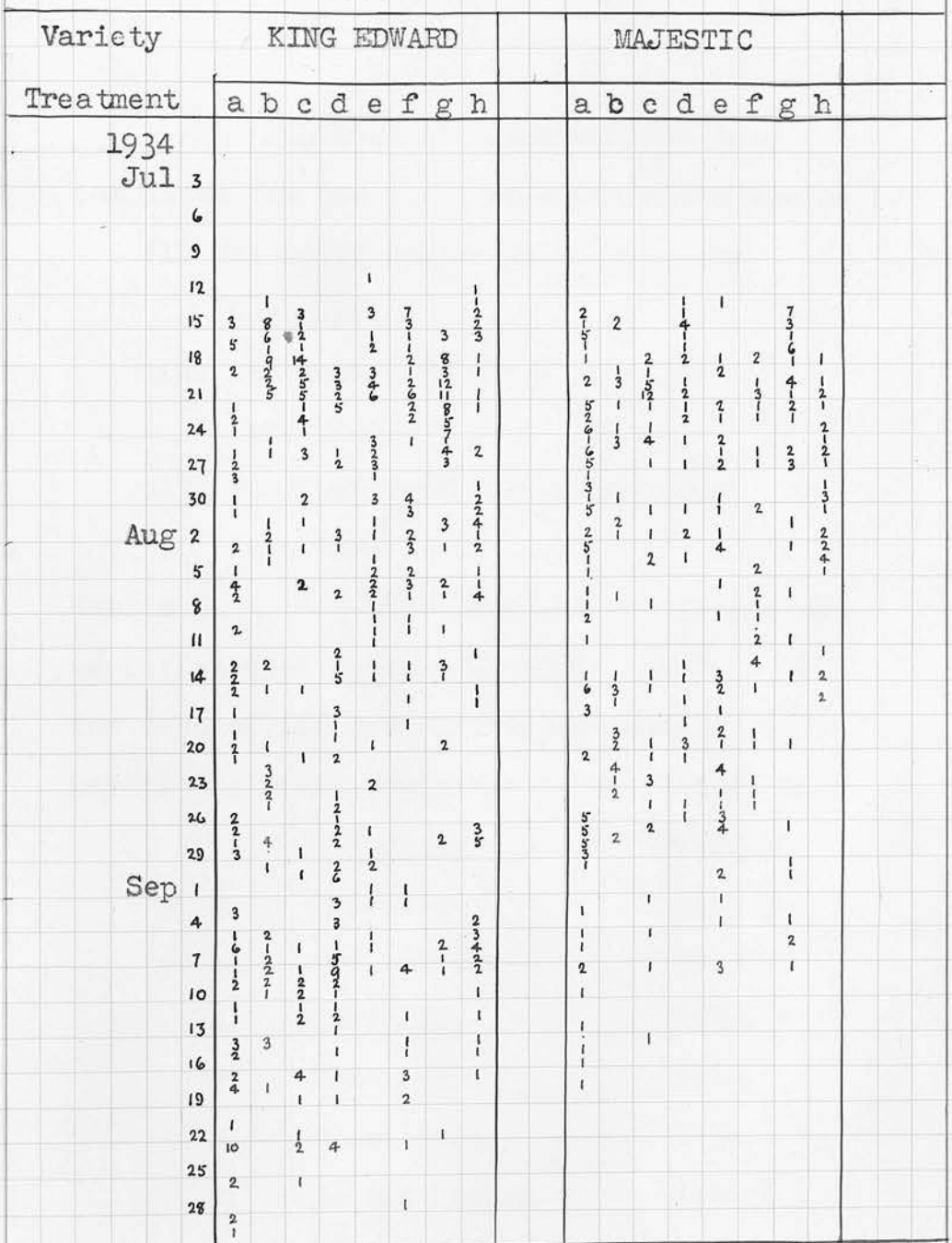


Fig. 97

Fig. 98

R E S U L T S.

Statistical analyses have been completed for the three undermentioned series :-

(1) The total number of flower-buds per plant.

(2) The number of flower-clusters per plant.

(3) The number of flower-buds per cluster.

Tables 3, 4, & 5 have been derived from eight varieties each containing eight plants - one for each of the manurial treatments. The key to the eight varieties is as follows :-

	Variety.	Season.
a.	Majestic.	1933.
b.	British Queen	1933.
c.	Catriona.	1934.
d.	Champion.	1934.
e.	Great Scot.	1934.
f.	Golden Wonder.	1934.
g.	King Edward.	1934.
h.	Majestic.	1934.

(1)./

(1) TABLE 3.

Total Number of Flower-buds per Plant.

Treatment	Varieties.								Total
	a	b	c	d	e	f	g	h	
PKN	118	205	154	228	96	152	167	114	1234
PK	41	56	46	102	20	83	88	39	475
PN	60	168	118	106	69	148	98	48	815
KN	83	172	84	137	32	156	108	31	803
P	23	90	47	116	25	84	69	50	504
K	33	74	90	108	30	56	79	30	500
N	64	137	155	133	52	102	120	47	810
No Manure	27	83	32	59	38	82	72	30	423
Total	449	985	726	989	362	863	801	389	5564

Analysis of Variance.

Variance due to :	D. F.	Sum of Squares	Mean Square	½ Log M.S.	Difference	Z.
Varieties	7	59500	8500	4.524	1.452	Points
Treatments	7	65837	9405	4.574	1.502	5%=.40
Residual	49	22841	466	3.072	-	1%=.55
Total	63	148178				

S.E. of single determination = 21.6
S.E. of total of 8 determins.= 61.1

Summary of the Effect of Various Manures on Total number of Buds per Plant.

Comparison	Aver. No. of Buds per Plant	Difference	S.E. of Difference.
Phosphate	95	16	30.5
No Phosphate	79		
Potash	94	14	
No Potash	80		
Nitrogen	114½	55	
No Nitrogen	59½		

(2) TABLE 4.

Number of Flower Clusters Per Plant.

Treatment	Varieties.								Total
	a	b	c	d	e	f	g	h	
PKN	10	15	11	19	8	10	18	10	101
PK	4	5	4	8	2	7	9	4	43
PN	5	12	11	8	7	10	8	5	66
KN	8	13	7	13	3	11	13	3	71
P	2	6	5	8	3	6	7	4	41
K	3	7	9	9	3	4	8	3	46
N	6	11	11	10	4	8	11	4	65
No Manure	3	7	5	5	4	7	8	4	43
Total	41	76	63	80	34	63	82	37	476

Analysis of Variance.

Variance due to :	D. F.	Sum of Squares	Mean Square	½ Log M.S.	Difference.	Z.
Varieties	7	340.25	48.61	1.952	1.370	Points
Treatments	7	374.5	53.50	1.990	1.408	5%=.40
Residual	49	157.0	3.20	.582	-	1%=.55
Total	63	871.75				

S.E. of single determination = 1.8
S.E. of total of 8 determins. = 5.06

Summary of the Effect of Various Manures on Number of Clusters per Plant.

Comparison	Aver. No. of Clusters per Plant	Difference	S.E. of Difference
Phosphate	7.84	.81	2.54
No Phosphate	7.03		
Potash	8.16	1.44	
No Potash	6.72		
Nitrogen	9.47	4.06	
No Nitrogen	5.41		

(3) TABLE 5.

Number of Flower-buds per Cluster.

Treatment	Varieties.								Total
	a	b	c	d	e	f	g	h	
PKN	24	28	28	24	24	30	18	23	119
PK	20	22	23	26	20	24	20	20	175
PN	24	28	22	26	20	30	24	20	194
KN	21	26	24	21	21	30	16	20	179
P	23	30	19	29	17	28	20	25	191
K	22	21	20	24	20	28	20	20	175
N	22	25	28	27	26	26	22	24	200
No Manure	18	24	13	24	19	24	18	15	155
Total	174	204	177	201	167	220	158	167	1468

Analysis of Variance.

Variation due to :	D.F.	Sum of Squares	Mean Square	½ Log M.S.	Difference	Z.
Varieties	7	423.25	60.46	2.051	1.117	Points
Treatments	7	207.00	29.57	1.693	.759	5%=.40
Residual	49	317.50	6.48	.934	-	1%=.55

S.E. of single determination = 2.55
S.E. of total of 8 determs. = 7.2

Summary of the Effect of Various Manures on Number of Flower-buds per Cluster

Comparison	Aver. No. of Flower-buds	Difference	S.E. of Difference.
Phosphate No Phosphate	23.75 22.13	1.62	3.56
Potash No Potash	22.75 23.13	- .38	3.56
Nitrogen No Nitrogen	24.13 21.75	2.38	3.56

Analysis of the daily flower-buds dropped by 48 plants (6 varieties each consisting of 8 plants - one for each of the manurial treatments) during a period of 65 days (July 16th. to Sept. 18th., 1934) gives the correlations shown in Table 6:-

TABLE 6.

Correlations between Flower-buds dropped and Mean Daily Temperature.

Temperature readings compared for:	Actual Correlation Ratios.	Level for Significance
Same day	.419	
1 day preceding	.413	(Number of pairs of observations N = 65).
2 days "	.391	
3 days "	.410	
4 days "	.445	
5 days "	.449	
6 days "	.373	
7 days "	.318	r = .32
8 days "	.393	
9 days "	.393	

This shows a first maximum at 5 days between temperature and the flower-buds dropped. Smith (1932) reported with tomato flowers and buds a lag of 3 days between temperature and it effect. He took daily counts on each of 10 plants for 38 days and showed the mean temperature figures for the same days.

Calculations/

Calculations by the present writer made on Smith's published data showed the following marked discrepancies in his correlation ratios :-

Correlations between Flower-buds dropped and Mean Daily Temperature of same and 4 preceding days.

Temperature readings compared for	Correlation ratios calculated by		Level for Signif.
	Smith.	Present Writer.	
Same day	.413	.454	
1 day preceding.	.476	.476	(Number of pairs of observations N = 35)
2 days preceding.	.499	.499	
3 days preceding.	.68	.597	
4 days preceding.	.63	.631	r = .44

Smith's claim of a maximum at 3 days cannot be substantiated by the present writer, who finds the correlation ratio still rising at 4 days.

Recalculation of further data published by Smith for the number of flowers formed and correlations with mean daily temperature revealed other gross errors as follows :-

Correlations/

Correlation between Number of Flowers formed and Mean Daily Temperature of same and 4 preceding days.

Temperature readings compared for	Correlation ratios calculated by		Level for Significance.
	Smith	Present Writer.	
Same day	- .21	.558	(No of pairs of observations - N = 35 to 39)
1 day preceding	- .139	.655	
2 days "	.113	.616	
3 days "	.499	.489	r = .44 - .41
4 days "	.435	.467	

The maximum in the present writer's correlation ratios is at 1 day, whereas Smith showed it at 3 days. The discrepancies are too great to be due to incidental approximations and Smith's ratios are at variance with the data given.

Continuing the examination of the present data on the dropping of potato flower-buds for other evidence of the effect of weather factors, the analyses show indications of greater flower-bud dropping :

(a) on the same day as the incidence of strong breezes where the plants were exposed to their action. This increase is probably due to the flowers actually being knocked

off/

off by neighbouring leaves and stems being blown against them by the breeze.

(b) on the same day due to changes in humidity. The variation in humidity shown in the weather chart is not as great as that which actually occurs, since the figures represent the humidity calculated from wet and dry bulb temperatures taken at 9 a.m.

The length of the pedicels of inflorescences in the experimental plants varied greatly from bud to bud in the same cluster, as well as from variety to variety and from treatment to treatment. A simple classification of the average length of the pedicels into groups Long (L), Medium (M) and Short (S) is given for 12 standard varieties in Table 7. The manurial treatments shown in the table consist of combinations of Superphosphate (P), Sulphate of Potash (K), and Sulphate of Ammonia (N). There are indications that Phosphate and Nitrogen treated plants have longer pedicels and that complete manuring also increases their length. It should be understood that the classification into Long, Medium and Short pedicels applies to the plant as a

whole./

whole.

Average Length of Pedicels.

Variety.	PKN	PK	PN	KN	P	K	N	No Manure.
Ally	M	S	M	S	M	M	M	S
British Queen	M	L	L	L	L	M	L	M
British Queen	S	S	S	S	M	M	M	M
Catriona	S	M	S	S	S	S	S	M
Champion	L	L	L	M	M	M	M	M
Di Vernon	M	S	M	S	S	S	S	S
Duke of York	M	S	S	S	S	S	S	S
Epicure	M	S	M	S	S	S	S	S
Golden Wonder	L	M	L	M	M	M	L	L
Great Scot	M	S	M	M	M	M	M	M
King Edward	L	L	M	S	S	M	S	S
Majestic	L	S	M	M	M	S	S	S
Majestic	M	M	S	S	S	S	S	S
Up-to-Date	M	S	S	M	M	L	M	M

L = Long.

M = Medium.

S = Short.

::::::::::::::::

D I S C U S S I O N.

The work reported in this section shows the very decided effect of manurial treatments on experimental plants. Indeed the high degree of statistical significance attaching to the number of clusters produced and the number of buds occurring in these clusters shows that work of this description is weakened, if not rendered valueless, if the fertility level of the soil used is not known with some accuracy for the various manurial ingredients.

The high degree of effectivenesss of nitrogen is not altogether surprising in view of the known requirements of flower initials. This too applies in the case of phosphate. The relatively poor increases in initials resulting from applications of potash, however, are interesting. As is well proven (Russell, 1932) potash affects the production and translocation of carbohydrates and shows its fullest effect when sunlight is deficient. The two summers in which this work was carried out had rather

higher/

higher sunlight figures than average. A repetition of the work in a dull summer might give somewhat different figures for potash than those shown here.

That varieties differ in their developmental characteristics, especially to manurial treatments, is well known. This has been worked out almost entirely for crop yield of tubers. Almost any report of varietal trials will show that varieties differ in their ability to respond to heavy manurial dressing. Put briefly the potential yield of a variety to excess manuring is conditioned by factors internal to the plant and there are some capable of exploiting heavy dressing and some not so capable. The same sort of thesis would seem to run through these figures.

The relationships of flower-bud drop to temperature, humidity and wind are also of interest in that they show significant responses to the impact of these factors. The 5 day lag in the response to temperature obtained for the dropping of potato flower-buds is similar to the only previous work published in this regard when

this/

this latter has been recalculated, and the now obvious inaccuracies removed. These responses have been obtained on plants exposed to a continually changing environment and they would probably be more marked in plants whose temperature and humidity were strictly controlled.

;::::::::::::

S U M M A R Y.

(1) The addition of fertilisers to the soil used definitely increased the number of flower-buds produced.

(2) The number of clusters in an inflorescence of potato and the number of flower-buds in each cluster is conditioned by the type of manurial dressing supplied to the plants.

(3) For the climatic conditions obtaining in Edinburgh during the seasons 1933 and 1934, nitrogen and phosphate are both capable of increasing the number of clusters and flower-buds in the cluster, nitrogen being rather more effective than phosphate. Potash had little significance.

(4) Varieties differ in the number of flower units produced.

(5) Varieties differ in the response of their flower primordia number to the various manures.

(6)/

(6) The conditions of temperature and humidity under which the plants are grown are proved to be active agents in abscission of flower-buds.

(7) The effect of mechanical agencies, for example, wind, in removing flower-buds is shown.

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PART III.

OPERATIONAL STUDIES ON THE VEGETATIVE REPRODUCTION OF POTATO TUBERS.

C O N T E N T S.

INTRODUCTION.

A whole field of study with an enormous literature has developed on the subject of the growth responses of the vegetative parts of plants. The vegetative propagation by natural modification (tuber, bulbs, etc,) or by artificial propagants (cuttings, grafts, etc.) has attracted considerable attention over a very wide field. On the more academic side the question of one plant unit composed of two or more components of different genetical or phylogenetic origin (chimeras) has received attention. Again the problem of the different behaviour of the proximal and distal aspects of an axial organ (polarity) is not without interest. In the potato all these aspects may be conveniently studied and in the work to be reported in this section they all bear in greater or lesser degree.

Describing the potato tuber Artschwager (1918) found that the eye in its entirety was a leaf scar with its subtended axil, which contained a suppressed lateral branch with several axillary buds and undeveloped internodes.

The /

The central bud of the eye was most prominent and developed first upon renewal of growth. Sections through the mature tuber showed several zones of tissue readily distinguishable to the naked eye. These zones were towards the exterior, the periderm, then a narrow cortex, then the vascular ring with a wide pith internally. Of these three areas the vascular tissue was least, the pith the most prominent. In the region of the "eye" the vascular tissue ran out in a lateral direction and provided the necessary vascular connection between the developing buds and the reserve materials stored in the tuber. The amount of the vascular tissue of the tuber was only slightly greater than that of the stolon, but the individual groups were much separated due to the expansion of the tuber, and it was only here and there that the vascular groups were united by inter-fascicular cambium. The xylem was mostly primary in nature, and only in the region of the larger groups were porous vessels of secondary xylem found. The phloem became broken up into small scattered strands. The cortex and pith differed mainly in the relative density of their

cells, the cortex being more dense on account of the smaller size of its cells and the larger amount of cell content other than starch. A periderm of 6 to 10 cells in thickness covered the entire tuber. The homogeneity of this layer was broken by small lenticel-like structures which were concerned with aeration of the underlying tissues. These lenticels as in the bark of other angiosperms were believed to have developed below the position of the stomata of the young stolon.

Artschwager (1924) described the potato tuber as "a modified stem with its axis greatly shortened and its lateral members only weakly developed, the latter forming what are known as the "potato eyes". The tuber originated as a stolon from an axillary bud of the stem typically underground. The eye borne on the tuber was morphologically a scale leaf scar with a suppressed lateral bud in the axil. The internodes were rather shortened and undeveloped. The eyes showed a definite spiral arrangement which was 13-ranked, since the 14th. eye was over the first after five turns of the spiral. Each eye contained at least three buds

arranged/

arranged in the form of an obtuse triangle and protected by more or less conspicuous scales. Often, however, a larger number of buds were present which then formed a secondary spiral to the left if the main spiral of the eyes was dextrose, or conversely if the latter was sinistrose." In ordinary vegetative propagation using whole tubers the rose end or terminal buds grew first and the growth of the others was inhibited. Denny (1928) showed that this inhibition of the laterals by the terminal bud in normal leafy branches as well as in the potato tuber could be countered by treatment with ethylene dichlorhydrin. The inhibition of the basal end eyes could also be removed by cutting off the rose end portion, as was widely practised in practical potato culture, in order to utilise large tubers more economically - 2 oz. being about the average weight of the sets generally recommended.

The simple operation of cutting the tuber with a knife starts a whole chain of somewhat complex reactions which affect many parts of the tuber. It is well known that lightly bruising the tuber is sufficient to

hasten the growth processes. In this connection Schlumberger (1926) claimed that tubers injured in digging, etc., germinated more quickly than uninjured ones. Even jolting or pressing the tubers had a noticeable affect. While cutting of the larger tuber at least is recommended in practice, care has to be adopted as secondary complications such as attacks of fungi on the exposed internal tissues may result. The sooner the surface heals the less likely are rot fungi to succeed in establishing themselves. To ensure quick healing of the wound in the cutting of the potato tuber, it is necessary to have high humidity and access of oxygen. Herklots (1924) showed the necessity for the presence of atmospheric oxygen for both suberisation and meristematic activity (callus formation). The wet bag system as advocated in Western Australia was used where possible in this work. The cut sets correctly marked with indelible pencil were placed in a wet bag made of coarse textured material immediately after the sets were cut, and the whole kept in the dark or shade for 24 hours. This provided ideal conditions for suberisation and callus formation.

Justesen/

Justesen (1931) found that the greatest cork formation on wounds occurred where the "mother" crop had been manured only with nitrates, the smallest amount of cork formation with those fertilised only with Potash and intermediate with complete manuring or none at all. Salaman (1926) and Priestley & Woffenden (1923) and Shapovalov & Edson (1919) showed that the protection afforded the cut surface of the potato by keeping the tubers in a warm moist atmosphere for from 24 to 48 hours was approximately equal to that of the skin itself.

Quite a considerable literature has been published dealing in one form or another with the subject of wound reactions in the potato, apart altogether from the larger subject of herbaceous plants. The work of Asseyeva (1927, 1930, 1931) on the chimera structure of certain potato varieties comes under this heading. This worker adopted the method of removing all the eyes of the tuber in order to stimulate the deeper lying tissues corresponding to the inner component of the chimera, the development of which provided a plant free of the outer component. Biffen (1902) investigating the effect of stock on scion and vice versa

found/

found no indication that transmission of characters occurred, with one exception, namely, when one variety of potato was grafted on another the result was that tubers were obtained with mixed tissues. This exceptional behaviour was thought to be due probably to the formation of a chimera.

Salaman (1930) proposed to limit regular chimeras to those where there was no doubt, from morphology and behaviour, of the relationship of the two or more component tissues. He classified these regular chimeras into three main types :-

(1) Periclinal - one tissue a complete envelope.

(2) Sectorial - sectors of each tissue reached centre.

(3) Mericlinal - one tissue enveloped a sector only.

The mericlinal chimeras were the most commonly found and were frequently formed from the growth at a graft union which had been cut back to expose both tissues. Salaman stated that mericlinal Solanum chimeras rarely remained long in that condition, but soon gave rise to

another/

another arrangement of tissues due to bud development and method of branching.

In the Dicotyledons the bud originates from one cell or, more usually, from a group of cells arranged in a transverse plane. According to Lange (1927) these cell initials were arranged in three or more transverse layers:

(1) The outer or uppermost layer gave rise to the epidermis.

(2) To the sub-epidermis.

(3) To the inner cortex and pro-cambium;

while (4) and (5) (in Solanum) also took part in the formation of the stele. The descendants of each cell were arranged in linear fashion in a longitudinal direction in the new shoot. As long as the bud initials remained of the same chimerical constitution, so long would the shoot remain constant in chimerical structure. However, buds often arose on the side of the shoot to form an axiliary branch, in Solanum the apparent main stem being made up of a number of branches, each of which gave rise to another by a fresh bud at intervals and, at the same time, stopped its own growth. This sympodial method of branching increased the opportunity for

variation/

variation in chimerical arrangement in one plant since a fresh bud was involved in development at each node. Where the arrangement of the tissues was periclinal in nature, external appearance might not be affected by this sympodial branching, but an opportunity was given for a doubling or reduction of the envelope layers. Hence it was possible to obtain one-layered periclinal from a two-layered and vice versa. Doubling and reduction of envelope layers in a periclinal chimera also took place by an unusual periclinal division in one of the cell initial layers of the original growing point. Lange (1927) has shown that such divisions did occur and has traced the anatomy of the process of layer formation. The reappearance of shoots composed of one "pure" tissue would be the final result of such a process. Mericlinals arising from periclinals in this manner were remarkably unstable. A figure drawn showed a periclinal chimera of Solanum containing four different and distinct tissues and thus it seemed that more than two layers did take part in stem and leaf formation in Solanums. Salaman (1930) found that the

difficulties/

difficulties of interpreting natural chimeras were increased by conflicting evidence regarding bud development. He considered bud-growth to be of two main types :-

(1) By two layers of cell initials.

(2) By more than two layers (as in Solanum).

The possibility of somatic segregation and/or polyploidy being induced by the wounding stimulus cannot be overlooked. Jorgensen & Crane (1927) were able to show by cutting back young vigorously growing plants of several species of Solanum that among the adventitious shoots arising from the callus, tetraploid and (in one case) triploid shoots were to be found. Rybin (1929) found that in the cortical tissue of the root of the potato it not infrequently happened that a whole section developed from a cell which had become tetraploid. Tetraploidy in somatic tissue must presumably have occurred through the failure of the developing cell wall to separate the two daughter nuclei after a normal mitotic division. A scarcely noticeable swelling of that portion of the rootlet involved was all that indicated what had occurred.

Salaman (1925) in examining the Arran

Victory/

Victory mutations of MacKelvie found that in the normal tubers the colouration of the surface was due to anthocyanin production in the superficial cortical layer; in some tuberscells may be found which are deficient in pigment. The amount of pigment varied from that giving a dark area to that giving a splashed or even colourless appearance. Sansome (1930) carried out a number of experiments planned to discover :-

(1) If mere operation on the tuber eye was sufficient to initiate a mutation in a stable variety.

(2) If so, did mutation occur in the field by reason of damage incurred as a result of breaking off of sprouts?

(3) Could mutation be induced in genetically splashed tubers?

(4) Were the Arran Victory mutations reversible?

As a result of operating by excision of the eyes on a great number of tubers of the varieties Arran Victory, King Edward and Di Vernon and growing on of the sprouts first formed before the eyes were excised, he came to the conclusion

that/

that mutation might occur but it was extremely rare. In Di Vernon the plants from operated tubers had healthy leaves, whereas in controls the basal leaves were abnormal. Certain cores which failed to produce sprouts produced minute tubercules on their cortical and medullary surface far removed from the excised eye. These tubercules were always suffused with purple pigment. This and other evidence showed that in the very heart of the Arran Victory tuber, whether externally coloured or not, the power to form purple pigment was still present. In one case, completely white tubers were formed in stolons growing out of the medullary tissue of a white mutant Arran Victory, which was taken as evidence that both colourless and colour-producing cells were present in the depths of the potato tissue. It seemed certain that the purple carrying tissue and the colourless one of a splashed tuber were not lying in an orderly manner as superposed layers, chimera-like, but were arranged in a highly irregular manner, now near the surface, now penetrating deeply into the tuber tissue.

Salaman/

Sansome (1930) further considered that it was just this disorderly-like arrangement of the tissues which accounted for the facts observed by himself and Asseyeva. The excision of the eye, whilst demonstrating the presence of an alternatively coloured tuber coat, almost invariably called forth a sprout bearing a mixed crop of mutant and type tubers, as would be expected if the tissue from which it sprang were made up of an irregularly mixed mass of mutated and non-mutated cells, the mutation being one of colour and not of pattern.

The mosaic theory put forward by Salaman in 1925 to explain the splashed tubers derived from the self coloured Arran Victory was slightly modified by Asseyeva's work. In Salaman's view the mosaic seen on the surface represented the tentacles of a mass of mutated cells lying in the deeper tissues, and that at certain given spots the actual disposition of the tissues might be of a more or less regular chimera, whilst a little further on it would depart from such. Dorst (1924) presented experimental data giving the results of planting eyes from sections of the same potato showing

differences/

differences in skin pigmentation. It was found that in the main an eye from a blue or yellow region gave rise to blue or yellow skinned tubers respectively in succeeding generations. He pointed out that while the observed cases of bud mutation had been confined to external morphological characters, easily discerned, nevertheless other character complexes such as quality, taste and starch content might also be modified in a like manner. A commercial variety contained a number of strains arising as bud mutations which differed considerably in their commercial value, some of which variations might be important in constructive selection.

Krenke (1933) subdivided chimeras according to causes leading to their production into ;-

(1) Graft chimeras.

(2) Stimulation chimeras.

(3) Hybridization chimeras.

He experimented with chimeras composed of Solanum lycopersicum and S. memphiticum, mostly with the tomato as the core. He found variation in the periclinal chimera grades, trichlamydius forms always showed poor growth,

dichlamydius/

dichlamydius forms were stronger, and monochlamydius forms showed greatest vigour. The leaf forms of the three grades showed differences, the greater the number of cell layers composing the skin the more nearly the leaf shape approached that of the species constituting the skin. He followed the differences between the tissues by histological features which in the species he used were very marked. Jorgensen (1927) unsuccessfully attempted to produce a chimera of potato having a skin of tomato, the purpose being to render the potato immune to infection by phytophthora. Winkler contemplated the theoretical possibility of chimera of two kinds of potato, the skin composed of a variety immune to phytophthora. Krenke is attempting to realise this possibility.

Jones (1934) summarised the types of chimera which might be distinguished :-

(a) Sectorial - very unstable and tended to change over to (b).

(b) Periclinal - very stable. Skin might be one or several layers thick - the ones being very constant the latter showing variations in thickness over different areas.

(c) Mericlinal - intermediate between (a) and (b) - frequently chimeras appeared as this.

(d)/

(d) Patterns - reversal of pattern sometimes occurred. Possession of a pattern was not in itself evidence of a chimera structure. Patterns were due to several causes and the component parts of all patterns did not differ genetically as did the components making up a chimera.

Chimerical structure has not yet been recorded in roots, but a number of instances appear in the literature showing that plants derived as root cuttings from periclinal forms were always composed of the component forming the core. The production of chimeras by any method will greatly assist in providing material for studying their structure and behaviour. Especially valuable are those methods which give chimeras synthesised of known components whose characters could be tested separately and in the combination. The possibility of using the starch-quality technique to test the presence of an inner component to a potato chimera where the tissues are otherwise indistinguishable is indicated. The known methods whereby chimeras are formed may be listed : 1. By placing the tissues of two different plants in contact so that they unite and grow as a single unit. 2. By a sudden and exceptional vegetative change or somatic change affecting a particular tissue

and /

and not the whole of an organ, both genetical types being perpetuated during subsequent vegetative growth. 3. By hybridization, graft hybrids, orderly vegetative segregation during development, high or low temperature, chemical agents, X-rays, etc. 4. By localised islands of anomalous tissues appearing. These are often merely local physiological effects and their nature must often remain obscure. If such areas represent a genetical difference in the tissues the chimerical structure is probably of local somatic change or somatic segregation.

The response of the potato tuber to wounding, cutting and various environmental conditions including invidence of disease factors, temperature, humidity, light, darkness, etc., has received some attention in the literature. McCullum (1905) found that the occurrence of regeneration in plants usually involved the replacement of parts removed, but the same result was often obtained when the organ was not removed, but prevented from functioning. A practical example of this in the potato was mentioned in the Bulletin of Ministry of Agriculture and Fisheries, the incidence of the

skin/

skin spot fungus (Spondylocladium atrovirens) which often succeeded in killing all the buds in all the eyes of the seed tuber, together with the meristematic tissue from which further buds in course of time often arose. The tuber itself was not necessarily killed, for in the course of the season it might produce a single hard wart-like callus at the scar at the heel end where it was originally attached to its stolon. Occasionally stalks would develop from adventitious buds on the wart, provided the tuber was given suitable conditions.

Numerous cases of the development of new potato tubers within old ones which had been stored over the summer have been recorded. Gager (1912) and Stewart (1918) both mentioned cases of this and showed that these new tubers were formed on ingrowing sprouts. Gager found that in many instances there was every indication of a reversal of polarity of the sprout. He thought it possible, although not always probable, that the ingrown sprout arose as a lateral branch starting at the very base of the main sprout and below the skin of the tuber. Detmer (1898) long ago called attention to the fact that the intensity

of polarity varied greatly with different species and was specially well marked in the case of the potato.

Isbell (1931) in studies on the regenerative capacities of leaf and shoot cuttings of potato found that the leaf cutting with axillary buds converted one of the axillary buds into either a shoot or a tuber. Stem cuttings of the potato converted one of the axillary buds into a tuber, tuber and roots, or a tuber in combination with the shoot and roots. In some cases roots were produced directly from the base of the cutting.

Callus formation in the potato tuber, such as reported in this paper, has not received attention in publications. Hopkins (1927) believed that activities leading to its formation in the potato consequent on wounding brought about increases in sugar content amounting to 53 - 68% of the original amount present. This sugar content began to increase immediately after injury, but reached a maximum in several days and then fell away.

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MATERIAL and METHODS.

For the operational work in the 1933 season 49 varieties of potatoes were obtained from the same sources as those mentioned in Part II of this paper. One tuber of each variety was grown as a control, whilst the other had its eyes and a small amount of tissue removed from half (if the tuber was cut in two) or from the whole tuber. The method has been briefly described by Asseyeva (1927). Six of the 49 varieties had coloured skinned tubers, while those of the remainder had white or brown russetted skins. In the 1934 season mostly coloured skinned varieties were used and the operational work varied as follows :-

(1) Removal of eyes with the minimum of tissue and damage to the tuber. This method did not allow of absolute certainty that the whole of each bud had been removed or allow of accurate delineation of the excised region.

(2) Removal of eyes to a known depth

regardless/

regardless of the amount of tissue affected. While this method exposed a large cut surface it was easy to gauge the depth of tissue removed in relation to the buds and a high degree of accuracy could be obtained.

(3) An attempt to secure plants grown from the eyes which had been removed and still get the deeper tissue in the eye region to sprout again. In these cases the operation work was delayed until the sprouts had reached a length of about 3", when they were broken off from the "mother" tuber and planted directly into pots of soil. The remaining eyes of these tubers were then removed with a minimum of damage to the tissue.

The seed stocks were marked with indelible pencil and treated soon after delivery by dipping in acidulated Mercuric Chloride solution (Mercuric Chloride 0.1% by weight, conc. Hydrochloric acid 0.5% by volume) for 1½ hours in order to control surface borne

fungus/

fungus diseases. Except where the sprouts were allowed to form the work of removing the eyes was started as soon as growth activity in the buds commenced. After being operated on the tubers were maintained in a moderately warm, moist atmosphere by storage in a wet, coarse-textured bag for at least 24 hours. They were then planted in moist sand contained in individual thumb pots, labelled, and the pots plunged in more moist sand in a large box.

At frequent intervals these tubers were examined and at the first sign of growth from the region of the eye the tubers were transplanted into larger pots of moderately rich loam, in which they were grown to maturity. No attempt was made to control the temperature of the tubers or plants, except that extreme heat or cold were avoided. Careful notes were taken on the vegetative characters at all stages of the plants produced and when the tops had died down the tubers were harvested, the crop weighed and other miscellaneous observations made.

In a number of special cases where the tubers were cut into separate portions it was noticed that callus growth occurred on certain

transverse/

PLATE 1. Callusing Test, showing "reconstructed" tuber.

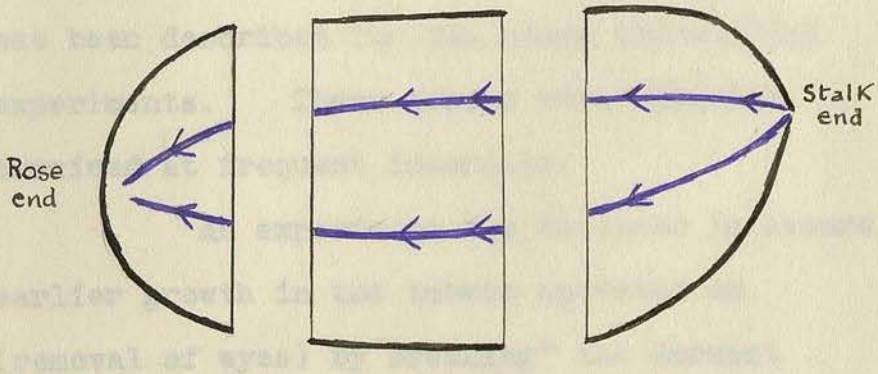

Diagram of "reconstructed tuber showing method of marking.

transverse faces, but was absent on all the longitudinal ones. There was an absence of callusing on the transverse faces proximal to the rose end. Hence, towards the end of the planting period of 1934 an experiment was carried out in which a number of tubers were cut to provide longitudinal and transverse sections. Some tubers were cut transversely into three sections (as shown in the illustrations), and others longitudinally. Before being cut the tubers were marked with lines and arrows from the stalk to the rose end by means of an indelible pencil, so that at any time the original tuber could be "reconstructed" from the pieces. In all these cases in addition to the division of the tuber, the eyes were removed to a considerable depth. The tubers were kept moist in a coarse bag for 24 hours and planted in sand as has been described for the other operational experiments. These tubers were likewise examined at frequent intervals.

An experiment was designed to secure earlier growth in the tubers operated on (removal of eyes) by "breaking" the dormant period to allow for the planting of both control

and/

and operational tubers to be made at the ordinary time. Some tubers were treated with Ethylene Chlorhydrin by dipping them in a 3% solution and then storing for 18 hours in a closed chamber in an atmosphere saturated with this chemical. With the unmutilated tubers this method was found to be effective in breaking the dormancy of the dormant tubers. No trouble was experienced before the work of cutting the treated tubers and removing the eyes was commenced. Then, in spite of provision of moist conditions for suberization of cut surfaces and clean sand for the next stage, rotting appeared in all cases. It was not ascertained whether or not this rotting was directly attributable to the Ethylene Chlorhydrin treatment.

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R E S U L T S.

The methods adopted in the work of operating on the potato with the removal of the eyes were so successful that hundreds of plants were obtained from shoots arising in the deeper - lying tissues. It was found that the method of removing the eyes to a known depth with consequent exposure of large cut surfaces was as good as that in which the eyes were removed with the minimum of damage, provided suitable conditions of moisture, etc., were maintained after the operation, and there was the advantage of being better able to gauge the depth and to remove each bud completely.

In the cases where the shoots were allowed to grow somewhat before the operational work was performed, the resultant plants from the shoots and the tubers did not produce such vigorous growth or as large a crop of tubers. Moreover, the work of removing the eyes was rendered unsatisfactory by the extensive growth of woody tissue at the eye region and the

difficulty/

difficulty of deciding whether the shoot formed in the deeper tissue came from the same bud initial as the original shoot, or from a suppressed lateral.

In 1933, the plants developed from the unmutilated tubers were so far advanced in growth ahead of those which had been operated on that comparisons of the vegetative characters were very difficult. In the varieties Rector, Golden Wonder, Arran Victory, Shamrock, Field Marshal, Goluboy, Krupnolistny and Alannah, the leaves of the operational plants were larger and more vigorous as compared with the controls in the same varieties. The operational plants in most cases were very late in appearing above the ground and this was probably responsible for any contrast in the appearance of the above-ground vegetative growth. The following varieties showed differences in skin colour as between the tubers produced from control and operated on tubers :-

VARIETY.	NORMAL.	OPERATED.
Reading Russet.	Pink, russetted.	Pale pink, smooth.
Golden Wonder.	Brown, russetted.	White, smooth.
Shamrock.	Pink.	White and pink, rather small & poorly developed.
Field Marshall.	White, russetted.	White, smooth.

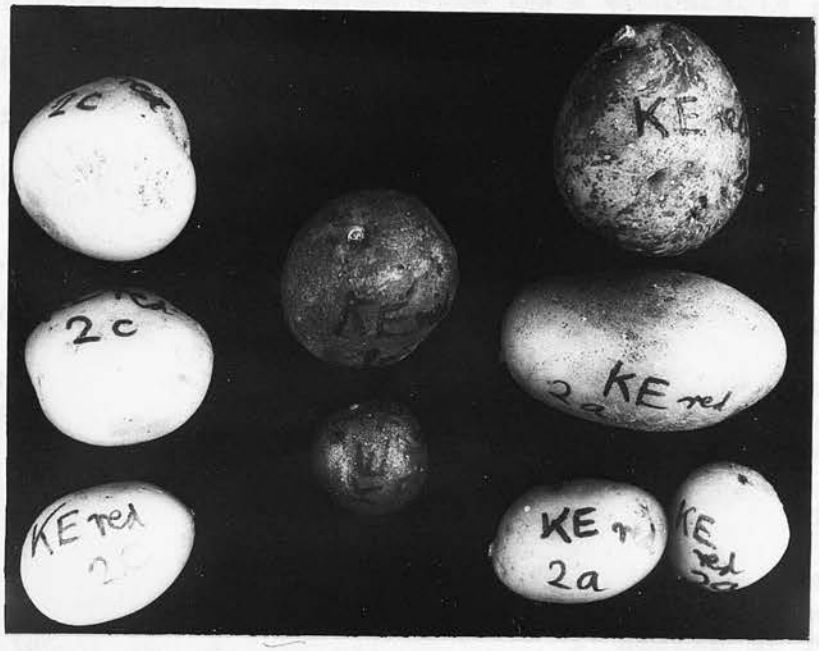

PLATE 2. Progeny of a Red King Edward tuber cut into three portions.

Left side : from stalk-end set from which the eyes had been removed.

Centre : control from middle set - not operated on.

Right side : from rose-end operated on for removal of eyes.

In 1934 no differences were observed in the foliage characters between the control and operated plants, which were more comparable as to age than in the previous year. In the tubers produced the following differences in character were obtained:-

VARIETY.	NORMAL.	OPERATED.
Golden Wonder	Brown, russetted.	White, smooth.
King Edward (Red)	Almost completely red.	One white, normal; and three with very little red.
" "	" " " "	All white tubers.
" "	" " " "	All white tubers, with minute specks of red. (No colour at the eyes).

The tubers from three portions of a Red King Edward tuber are shown in Plate 2. The two tubers in the centre of the photo are from the middle, or control, portion of the original "mother" tuber; the yellow tubers at either end are from plants produced from shoots arising from the end sets whose eyes had been removed. The occurrence of one normal tuber in the four from the rose end set is shown. There is no evidence in this case for or against its differential origin.

The/

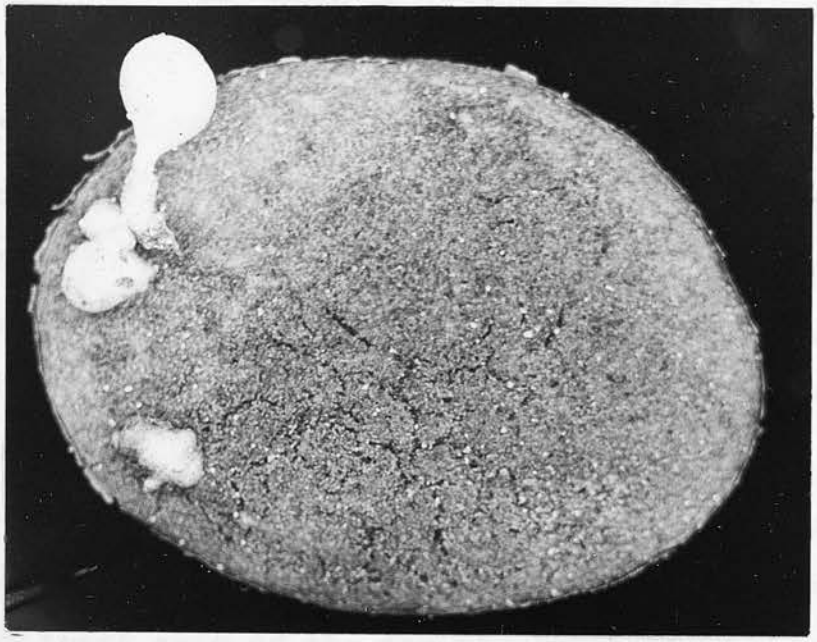

PLATE 3. Cut face "operated on" tuber of Arran Victory, showing proliferation on vascular band and formation of new tuber from the deeper tissue.

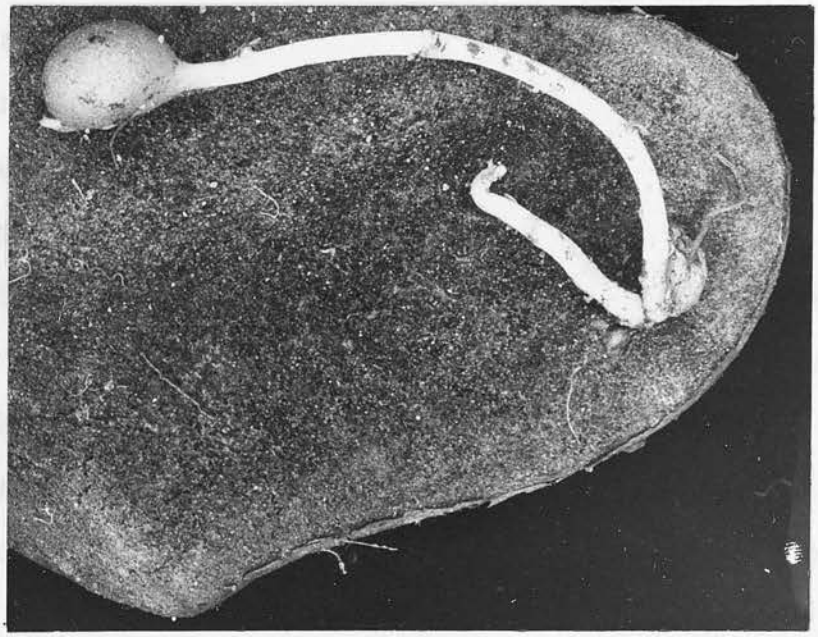

PLATE 4. Cut face "operated on" tuber of Kepplestone Kidney, showing similar proliferation to that of Plate 3, but with formation of tuber and shoot.

The work on callus formation in cut tubers whose eyes had been removed revealed the fact that shoots may be secured in the deeper tissue and away from the influence of the eye with its "nest" of buds. In a large number of such cut sets growth at the eye region was delayed or inhibited, but active proliferation took place on the larger cut surfaces of the sets, mainly on the vascular band, as seen in the middle tuber of Plates 3 and 7. In one of these sets of the variety Arran Victory where proliferation was proceeding well inside the original periderm of the potato, there appeared suddenly a bud which developed and soon became clavate forming a small complete tuber, whitish in colour, at the end of a short stolon. This is clearly seen in Plate 3. The partial break in the stolon visible in this photograph occurred accidently just before the plate was exposed and further growth could not take place.
Microscopic sections of the stolon and tissues of the callus were made in an attempt to trace the origin and organisation of the stolon. A smaller growth of shoot and tuber took place in the variety Kepplestone Kidney. This is shown

in/

PLATE 5. Shoot arising from callus growth on cut surface of "operated on" tuber - variety King Edward.

in Plate 4. The beginning of a third instance occurred on the middle section on a tuber of the variety King Edward used in the callusing test, the shoot only being formed. This was removed at the stage shown in Plate 5, and sectioned to trace its connection with the original tissue of the tuber. The photograph was taken at a tangent to the cut surface to show that the shoot arose from a point in the proliferating tissue, and not direct from the vascular region of the tuber. The shoot exhibits negative geotropism since it has turned towards the cut surface, which was placed downwards in the moist sand.

Among the examples of this proliferation on the vascular band of the cut surfaces numerous cases were observed where this had a definite polarity occurring on the one transverse cut of the set and not on the other transverse cut or on the longitudinal ones. Where the cuts were diagonal there was some callusing on the one face. In the tubers specially cut for observation of this peculiar localisation of callusing there were transverse cuts forming three sections, (marked by indelible

pencil/

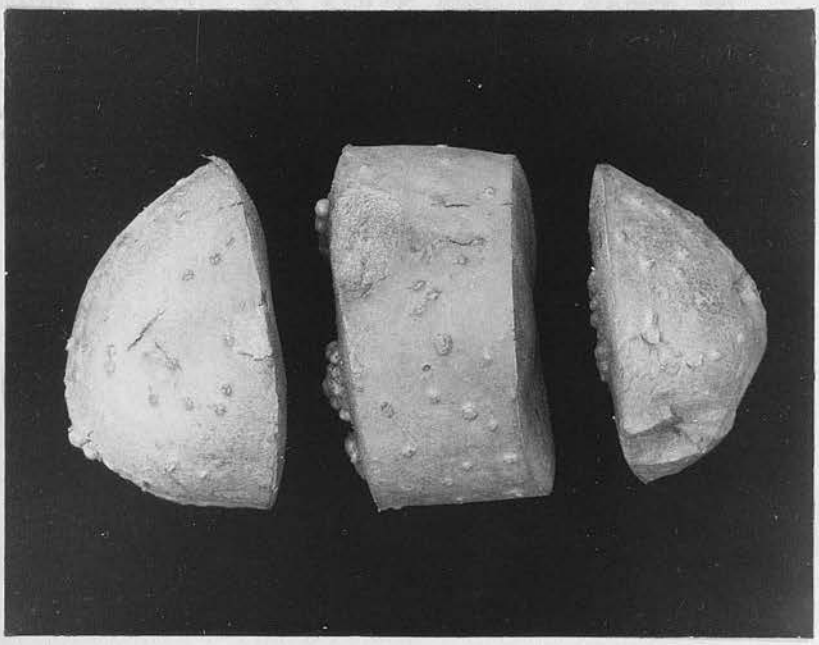

PLATE 6. "Reconstructed" tuber of variety King Edward showing definite polarity of callusing growth.

Left side : stalk end set showing callus growth at the stalk only.

Centre : middle set showing callus growth on face towards stalk end.

Right side : rise end set showing callus growth on face towards stalk end.

All three sets had the eyes removed.

pencil with lines to allow the tuber to be "reconstructed") callusing took place on the faces towards the stalk end, as shown in Plates 6 and 7, and not on the other faces, Plates 6 and 8.

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PLATE 7. View of stalk-end & cut surfaces showing presence of callus growth on vascular band - variety King Edward.

PLATE 8. View of rose-end and cut surfaces of other sets facing away from stalk end showing absence of callus growth - variety King Edward.

D I S C U S S I O N.

There seems no doubt that in the potato chimerical conditions exist in a number of so-called varieties that such a standard well known commercial sort as "Golden Wonder" may be resolved into two distinct components - Golden Wonder and a smooth white type similar to "Langworthy" is deserving of notice. From the genetical point of view this opens up two main questions, (a) is clonal selection and the search for "bud sports" worth while ?, and, (b) which of the two (or possibly three) components in a variety will be responsible for gamete production?. A subsidiary question might be, "Does more than one of the components give rise to the gametes in one and the same plant?"

The first question is not easily answered, for as can be seen from the tables furnished, no "new and improved" variety came from the operated on tubers. In this regard, however, it must be remembered that no physiological tests (yield, quality, disease resistance, etc.) were made on the extracted

component/

component. The research up to this report has been kept simple by referring only to easily recognised characteristics. This will not be sufficient, however, in the future and the physiological results of the interaction of component upon component in the one unit will have to be compared with the characters of the individual components. For example, Langworthy a high quality variety, has been shown to be responsible for all the tissue below the periderm of Golden Wonder, another high quality variety - how far is the whole of the quality ascribed to Golden Wonder due simply to Langworthy? When the superficial component is isolated will a low quality golden skinned variety eventuate? Furthermore, if as is believed, the more superficial tissues are concerned in gamete formation the use of "Golden Wonder" (commercial variety) as a quality parent in hybridisations may be entirely wrong, "Langworthy" being better. Thus the production of a genetically golden skinned high quality potato would seem to be possible only by crossing the golden skinned component with the white skinned component and isolating the segregant.

This/

This aspect is not a matter of practical politics so long as the chimerical Golden Wonder does not break down under field culture conditions. As soon as white skinned "mutants" or sports commence to appear in the field the whole matter will become of practical interest in view of the demands by modern legislation (Regulations under "The Seeds Act, 1920") for very high purity as a sine qua non for seed stocks. That this breakdown of a chimera under field conditions is possible and does occur is evidenced by the findings of MacKelvie in the variety Arran Victory, and also by the "White Kerr's Pink" which arose as a bud sport in Kerr's Pink in the field. (Private communication from Mr. Kerr).

The question of the possible value of "bud sports" then would seem to turn on empirical methods in the present state of our knowledge. Their value (given that they are the result of chimerical separation) must turn on the genetical constitution of the exposed component and this can only be ascertained by breeding experiments and there is no reason to believe that such would be different from a simple variety similarly bred. In addition to this reliance on

genetical/

genetical constitution the possibility of a component behaving differently when in a chimera than when isolated must too be cleared up.

The results from callusing show that the orientation of the cut surface to the whole organ is of major importance. The work of Went (1935) on the transmittal of auxin or growth hormone might be invoked in this regard. Went and others have shown that the hormone responsible for the growth reactions on the coleoptile of the oat, etc., can only move downwards. In the potato the requirements would be that it move from the morphological base towards the apex. The cases are not parallel, however, as Went's auxin is responsible for cell expansion through cell wall extension, while callusing must certainly be regarded as cell proliferation. If the finding of Snow (1933) and co-workers that upper buds inhibit lower buds, but that lower buds are ready to start growth promptly on the inhibition being removed be accepted, then the same sort of thesis could be offered for the fact that cut faces of the cut portion distal from the area nearest the apex

proliferate and grow. The activity of the cut surface could then be ascribed to the stimulus of cutting coupled with the removal of the inhibition of the upper potentially inhibiting tissue. The fact that these active callus tissues produced buds is not surprising in view of the general experience of workers in plant propagation. The mechanism whereby the bud was organised by non-organised tissue is, as in the general case, obscure.

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S U M M A R Y.

(1) Operation on potato tubers by excising the eyes shows that some varieties at least are chimeras.

(2) The chimeras noted have components showing morphological differences.

(3) The possibility of chimeras showing no morphological differences, but physiological differences, is mooted.

(4) The practical significance of chimeras on quality, plant breeding results, etc., is noted.

(5) The possible effect of component on component to give a variety qualitatively different from either by virtue of their reactions on each other is noted.

(6) Callusing of the cut surface of a potato tuber shows polarity. Cut surfaces facing towards the stalk end callused freely, while the faces on the opposite aspect did not.

(7) Tubers cut along a line on or parallel to the morphological axis did not callus.

(8) Such free callus may produce fully organised buds over the cambial area of the tuber vascular bundle.

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